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The impact of 2 weeks detraining on phase angle and muscle strength in trained older adults

Dissertação elaborada com vista à obtenção do Grau de Mestre em

Exercício e Saúde

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Abbreviations

ALM	appendicular lean mass
BCM	body cell mass
BF	body fat
BIA	bioelectrical impedance analysis
BIS	bioelectrical impedance spectroscopy
BMC	body mineral content
BMI	body mass index
CVD	cardiovascular disease
ECW	extra-cellular water
FFM	fat-free mass
FM	fat mass
GTO	golgi tendon organs
H	height
h/d	hours per day
ICW	intra-cellular water
L	liters
LIPA	low-intensity physical activity
m	meters
MF-BIA	multi-frequency bioelectrical impedance analysis
MS	muscle strength
MV	muscle volume
MVPA	moderate-to-vigorous activity
PhA	phase angle

R	resistance
SB	sedentary behaviour
SF-BIA	single frequency bioelectrical impedance analysis
TBW	total body water
Vo2 max	maximum rate of oxygen consumption
XC	reactance
Z	impedance

Abstract

The impact of 2 weeks detraining on phase angle and muscle strength in trained older adults

An interruption in the training routine may have deleterious effects on muscle performance and bioelectric markers, namely phase angle (PhA), which is a predictive marker for cellular integrity.

Purpose: To determine the effects of a 2-week detraining period on PhA and MS in older trained adults.

Methods: A total of 14 older adults (6 females) aged ≥ 65 years (77.2 ± 6.6) were assessed at baseline (i.e. trained) and after 2 weeks of detraining, for PhA and MS. PhA (º) was assessed using bioelectrical impedance analysis (BIA). MS was assessed on both lower and upper limbs under isometric conditions. Differences between moments were examined using the general model for repeated measures, with adjustment for sex. Statistical significance was set at $p < 0.05$ and a power of 0.80 for all the analysis.

Results: Detraining resulted in declines in PhA ($p = 0.017$). No differences were found in MS, for both leg press ($p = 0.992$) and bench press ($p = 0.166$) assessments. One association was found between PhA and bench press ($r = 0.76$, $p<0.05$), in post-detraining.

Conclusion: A short-term detraining period of 2-weeks resulted in detrimental changes in PhA but not in MS. These results highlight the importance of maintaining structured exercise sessions in older adults.

Key words: Physical inactivity; detraining; phase angle; muscle strength; isometric testing; BIA; older adults

Resumo

Efeitos de 2 semanas de destreino no ângulo de fase e força muscular em idosos treinados

Uma interrupção da rotina de treino poderá ter consequências negativas na performance muscular e em marcadores bioelétricos, nomeadamente no ângulo de fase, que é um indicador de integridade celular.

Objetivo: Investigar os efeitos de um período de 2 semanas de destreino no ângulo de fase e na força muscular em pessoas idosas treinadas.

Métodos: Um total de 14 pessoas idosas (6 mulheres) com idades igual ou superior a 65 anos (77.2 ± 6.6) realizaram uma avaliação num momento inicial e após 2 semanas de destreino para determinar o ângulo de fase e a foça muscular. O ângulo de fase foi avaliado através da análise por bioimpedância (BIA). A força muscular foi medida nos membros inferiores e superiores, sob condições isométricas. As diferenças entre momentos foram analisadas através do modelo linear geral para medidas repetidas, controlado para a covariável sexo. Foi assumida uma significância estatística de $p<0.05$ e uma potência de 0.80 para todas as análises realizadas.

Resultados: O destreino resultou na diminuição do ângulo de fase ($p=0.017$). Não se observaram diferenças na força muscular, tanto na prensa de pernas ($p=0.992$) como no supino ($p=0.166$). Apenas foi encontrada uma correlação positiva entre o ângulo de fase e a força de supino ($r = 0.76$, $p<0.05$), no momento pós-destreino.

Conclusão: Um período de destreino de curta duração (2 semanas) promove alterações no ângulo de fase, mas não na força muscular. Estes resultados destacam a importância da participação regular de populações idosas em sessões de exercício estruturadas para se preservar a integridade celular.

Palavras-chave: Inatividade física; destreino; ângulo de fase; força muscular; métodos isométricos; bioimpedância; idosos

Introduction

People have been spending more time in sedentary behaviours (SB), essentially sitting (Hamilton, Hamilton, & Zderic, 2007), which has been highly influenced by industrialization and financial growth because of the shift in the type of activities that people perform in their daily lives (Hill, Wyatt, Reed, & Peters, 2003; Lanningham-Foster, Nysse, & Levine, 2003). This is considered a global trend which is likely to continue and cause several negative effects, such as the increment of body adiposity and impairments in physiologic markers (cholesterol, triglycerides, blood glucose) which contribute to the increase of the risk of metabolic and cardiovascular diseases and some cancers (Bowden Davies et al., 2018; Hamilton et al., 2007; Katzmarzyk, Church, Craig, & Bouchard, 2009). Specifically older adults, when compared to adults, spend more time in SB, which may be particularly concerning given all the harmful effects of aging (A. E. Bauman, Petersen, Blond, Rangul, & Hardy, 2018).

With the increase of age, several changes occur on some of the major physiological and biological systems of the body. These age-related changes may be pathological and lead to a dependent state in which the older adult may have poor mobility, weakness, increased risk of falling, fractures or even diseases, such as osteoporosis or osteoarthritis (Freemont & Hoyland, 2007). The magnitude of these pathologies may be aggravated when elderly adopt SBs and refrain from physical activities.

Only 35% of the older people reach the recommendations for 150 minutes/week of at least moderate physical activity, with a prevalence of 46% for men and 29% for women (Baptista et al., 2012). Participating in exercise programs, specifically for the elderly, enhances several capabilities and helps provide more years of mobility, independence, and better quality of life by escaping from disability to perform activities of daily living (Fatouros et al., 2005; Faulkner & Brooks, 1995). Although exercise is not capable of preventing the inevitable decline in the functional capabilities of skeletal muscle with aging, it will at least delay the

aggravation on quality of life (Faulkner & Brooks, 1995). Therefore, it seems crucial to maintain an active lifestyle with regular practice of exercise and to avoid, as much as possible, long periods of inactivity (Dos Santos, Cyrino, Antunes, Santos, & Sardinha, 2016).

Although the benefits of exercise are well known, there is little evidence on the damages induced by the detraining. Detraining seems to promote a partial or complete loss of training-induced physiological and anatomical adaptations as well as a loss in performance (Fleck, 1994; Mujika & Padilla, 2000). The magnitude of these deleterious consequences is still not well understood in older populations.

Some of the markers affected by the combination of aging, SB and physical inactivity concern bioelectrical properties - such as phase angle (PhA) -, and muscle strength (MS), which will be the focus of the present thesis.

1. Bioelectrical Impedance Analysis (BIA)

1.1. Definition and Principals

Bioelectrical impedance analysis (BIA) has been frequently used in diverse clinical situations, so it seems important to be aware of its principles and methods and also the body compartments that it assesses (Kyle et al., 2004).

BIA is a widely used method to measure passive electrical characteristics in living organisms, providing the results rapidly. It is known for being a safe, simple, portable, non-invasive and reliable method, which uses biophysical and regression models to predict body composition, since it does not directly determine it. BIA devices induce a constant, low-level alternating current thought the placement of a tetrapolar surface electrode on both hands and/or feet, providing whole-body values (Kyle et al., 2004; Lukaski, 2013; Lukaski, Kyle, & Kondrup, 2017). BIA devices may be phase-sensitive or non-phase-sensitive. Phase-sensitive impedance uses a single frequency of 50 kHz, which allows the direct measurement of PhA

and impedance (Z), and calculates reactance (Xc) and resistance (R) through a trigonometric approach (Sardinha, 2018). The non-phase-sensitive devices do not provide the frequency-dependent phase-shift and use a range of frequencies to generate the best fit model from data acquisition at each frequency in order to calculate Z and PhA (Bosy-Westphal et al., 2017; Sardinha, 2018; Ward, Essex, & Cornish, 2006). This approach may not be accurate when calculating PhA (Sardinha, 2018).

The impedance in BIA is affected by specific frequency, body geometric shape and cross-sectional area. Therefore, fat-free mass (FFM), which includes a protein matrix and contains most of the water and conducting electrolytes in the body, has a greater conductivity when compared to fat mass (FM) (Lukaski, 1987). Intracellular and extracellular fluids are rich in electrolytes and, therefore, are greater conductors of electricity than cell membranes, which have a phospholipid bilayer structure. While low frequencies pass only through extracellular fluids, higher frequencies are able to penetrate cells and access intracellular fluids (Lukaski, 1987; Lukaski, Bolonchuk, Hall, & Siders, 1986).

BIA enables the determination of the quality of cellular membranes and the body fluid distribution, identifying biomarkers of cellular damage or dead cell in geriatric populations (Lukaski et al., 2017).

1.2. Types/Methods of BIA

There are several methods based on the principles of bioelectrical impedance, classified in the following major categories: single frequency BIA (SF-BIA), multi-frequency BIA (MF-BIA) (Kyle et al., 2004), bioelectrical impedance vector analysis (BIVA) (Piccoli, Rossi, Pillon, & Bucciante, 1994) and specific BIVA (Marini et al., 2013). For the purpose of this study, SF-BIA and BIVA were used.

1.2.1. Single frequency BIA (SF-BIA)

SF-BIA is a method based on mixture theories and empirical equations. It utilizes a frequency of 50kHz, which passes between surface electrodes placed on the hand and foot (or had-to-hand, or foot-to-foot), measuring the weighted sum of extra-cellular water (ECW) and intra-cellular water (ICW) resistivities (~25%), in order to estimate total body water (TBW). However, this method cannot detect differences in ICW. SF-BIA is not valid under conditions of significant dehydration and tends to significantly underestimate TBW and overestimate body fat in healthy individuals (Kyle et al., 2004; Martinoli et al., 2003). SF-BIA is also capable of estimating fat free mass (FFM), which is described as "everything that is not fat". It can be determined by gender, age and ethnic group specific SF-BIA equations, assuming that hydration is normal (Kyle et al., 2004).

1.2.2. Multi frequency BIA (MF-BIA)

MF-BIA is a device that uses regression models and performs its analysis at two types of frequencies: one at very low frequencies (normally 5kHz) and the other at higher frequencies (50, 100, 200 to 500 kHz) (Kyle et al., 2004; Thomasset, 1962; Ward, 2019). The impedance data are applied to regression-derived equations in order to predict TBW, ECW, and ICW (C. Earthman, Traughber, Dobratz, & Howell, 2007).

The bioelectrical impedance spectroscopy (BIS) is an approach of MF-BIA that measures across a spectrum of frequencies and uses mathematical modelling and mixture equations to generate relations between R and body fluid compartment (Kyle et al., 2004). The spectrum follows the model of Cole-Cole (S. Cole & H. Cole, 1941), which allows the existence of theoretical impedances at the frequency of zero and infinite. BIS has the advantage of only using low frequencies through the ECW, while it uses high frequencies (> 50 kHz) to flow through both ECW and ICW (Ward, 2019). Therefore, it provides a more direct,

individualized measurement of ECW and ICW than other impedance approaches (C. Earthman et al., 2007). It also estimates TBW, as the sum of ECW and ICW, fat free mass and fat mass. It is valid for all populations, regardless their health status, age, ethnic, or other conditions (Patel, Matthie, Withers, Peterson, & Zarowitz, 1994). The values of impedance measured in a certain spectrum of frequencies may explain variations in the body composition between individuals more precisely than single frequency devices.

1.2.3. Bioelectrical impedance vector analysis (BIVA)

BIVA is an integral part of BIA and combined, they provide a valuable evaluation of body composition in humans, although this method does not measure body compartments quantities (Norman, Stobaus, Pirlich, & Bosy-Westphal, 2012; Walter-Kroker et al., 2011). BIVA, developed by Piccoli, is a simple and quick method that uses impedance measurements, such as R and reactance (Xc), to access fluid status (i.e total body water) and body cell mass (BCM) (Bosy-Westphal, Danielzik, Dorhofer, Piccoli, & Muller, 2005; Piccoli, Rossi, Pillon, & Bucciante, 1996; Walter-Kroker et al., 2011). It uses a bivariate vector as a nomogram in the RXc mean graph and it`s normalized per subject`s height (H) (average of R/H and Xc/H, measured in Ohm/m) (Bosy-Westphal et al., 2005; Piccoli et al., 1994; Walter-Kroker et al., 2011). The standardization for H is important because it provides a qualitative measure of soft tissue regarding body size (Norman et al., 2012; Piccoli et al., 1994).

Because there are differences in the vector distribution patterns, it is necessary to define reference distributions of impedance vectors (R and Xc) accordingly to sex, race/ethnicity, BMI, and age stratifications (Bosy-Westphal et al., 2005; Piccoli et al., 1995b; Piccoli, Pillon, & Dumler, 2002)

In the RXc graph the reference values are plotted in three specific tolerance ellipses in the same coordinate system, corresponding to the 50^{th}, 75^{th} and 95^{th} vector percentile of the

healthy population (Piccoli et al., 1995b; Piccoli et al., 2002; Piccoli et al., 1994; Walter-Kroker et al., 2011). After all the adjustments, the position and the length of the vector gives information about an individual`s hydration status (length of the vector) and BCM and integrity (migration sideways) (Bosy-Westphal et al., 2005; Norman et al., 2012; Piccoli et al., 1994; Walter-Kroker et al., 2011). Values of healthy individuals are usually within the 75th tolerance ellipse (Norman et al., 2012; Piccoli et al., 1998) and values located outside the 95th percentile are considered abnormal and may signal different health conditions depending on the quadrant they are located in (Bosy-Westphal et al., 2005; Dorhofer & Pirlich, 2005; Walter-Kroker et al., 2011): exsiccosis/dehydration, characterized by increased R and a longer and upper vector (right upper quadrant); oedema/fluid overloading, decreased R, shortening and down-sloping of the vector (left lower quadrant); malnutrition and BCM decrease, low Xc (right lower quadrant); and good training status and BCM increase, high Xc (left upper quadrant) (Figure 1) (Dorhofer & Pirlich, 2005; Piccoli et al., 1994; Walter-Kroker et al., 2011).

Also different types of individuals present a pattern for different bioelectrical characteristics: obese present high PhA and short vector; athletes present high PhA and long vector; cachectic present low PhA and long vector; and lean individuals present normal PhA and long vector (Norman et al., 2012).

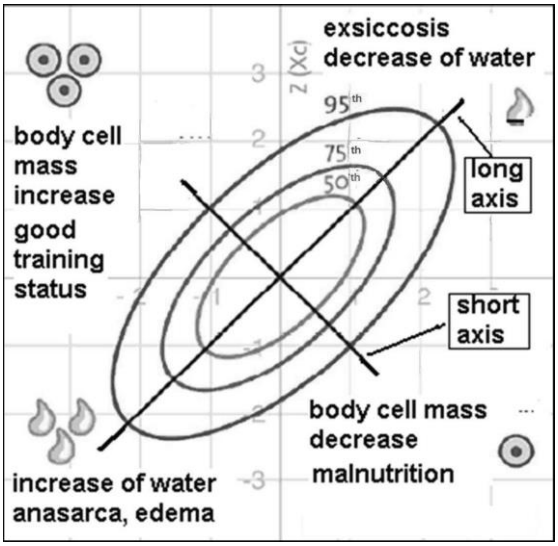

Figure 1: *Interpretation of the BIVA nomogram (Walter-Kroker, Kroker, Mattiucci-Guehlke, & Glaab, 2011)*

BIVA has shown to be an effective method to access health status and identify several conditions, such as renal pathologies, liver disease, obesity, cachexia, and anorexia (Buffa, Floris, & Marini, 2003; Piccoli et al., 2002; Piccoli et al., 1996). Also BIVA seems to be important to identify quantitative changes of body composition in the elderly, allowing the perception of nutritional variations, reduction of soft tissue and possible pathologic conditions during the aging period (Buffa et al., 2003; Norman et al., 2012). Particularly after the age of 80, for both men and women, soft tissue mass and its electrical properties tend to decrease, leading to a reduction of the capacitive component (Xc/H), as well as PhA (Buffa et al., 2003; Norman et al., 2012). This is due to the quantitative and qualitative changes of the lean and fat tissues of the body (e.g. reduction in the number of muscle fibres and motor units, particularly in the limbs, highly associated with sarcopenia) (Buffa et al., 2003; Norman et al., 2012). The values of R/H tend to either stay normal or increase (Buffa et al., 2003).

Compared to PhA alone, BIVA enables a better understanding of BCM integrity and hydration status. This two methods used together allow a better monitoring and possible

identification of patients at risk, concerning hydration and nutritional status. According to the R and Xc, theoretically, different positions of the vector in the RXc graph produce similar PhA values (Norman et al., 2012).

1.2.4. Specific BIVA

Marini et al. (2013) developed an additional analytical variant based on the classic BIVA: the specific BIVA. This alternative has proven to be an effective and practical method to evaluate body composition since it uses the same approach as classic BIVA and easily calculates specific bioelectrical values from bioelectrical and anthropometric parameters, regarding body geometry (Marini et al., 2013). In specific BIVA, R and Xc are used with the same vectorial approach of classic BIVA, however these impedance values are not only standardized for body height, but also for body volume, in order to estimate the whole-body impedivity vector (Marini et al., 2013; Saragat et al., 2014). To enable this, R and Xc values are multiplied by a correction factor, Area/Length (meters, m), using estimated values of Area and Length: "Area = (0.45 * arm area + 0.10 * waist area + 0.45 * calf area) (m^2), where segment area = C2/4JI, and C (m) is the circumference of the arm, waist and calf, respectively; Length = 1.1 H, where H is body height in meters" (Marini et al., 2013). These new bioelectrical variables represent the impedivity or specific impedance, which is the square root of sum of squares of resistivity and reactivity. Specific R and Xc are multiplied by a factor of 100 to obtain the same order of magnitude as classic BIVA (Marini et al., 2013).

Resistivity and reactivity provide an indicator which is positively associated with the relative fat mass, detecting different quantities of body fat in individuals with similar BMI (Buffa, Mereu, Succa, Latini, & Marini, 2017; Marini et al., 2013). The lengthening of the impedivity vector indicates an increase in FM% values (Marini et al., 2013).

Specific BIVA may be an interesting method to use during the monitoring of sarcopenia and sarcopenic obesity (age-related conditions), since it seems effective in identifying changes in the body composition of older people (Marini et al., 2013). It also seems to be more accurate than BMI when accessing FM%, so it would be useful to screen obesity and, especially normal-weight obesity (Buffa et al., 2017).

1.3. Phase Angle (PhA)

1.3.1. Definition and reference values

PhA is a raw parameter measured in degrees (º) by BIA which is directly affected by changes in the amount and quality of soft tissue mass (Bosy-Westphal et al., 2006; Tomeleri et al., 2017). PhA depends on Xc, which includes cell size and integrity of the cell membrane, and R, such as tissue hydration. Therefore, mathematically, PhA is obtained from the arctangent of the Xc to R ratio ((Xc/R) x 180º/π) (Bosy-Westphal et al., 2006; Tomeleri et al., 2017). R and Xc will be discussed in more detail in topic number 1.4.

PhA provides information about the cellular health of the whole body and has been used as a crucial biomarker of several clinical conditions (Gonzalez, Barbosa-Silva, Bielemann, Gallagher, & Heymsfield, 2016). PhA normal values depend on the population group, which constitutes a disadvantage since its range is very wide, limiting comparisons among groups (Grundmann, Yoon, & Williams, 2015). There is evidence that higher values for PhA (>6.6º) are associated with a greater amount of intact cell membranes and therefore, a longer life span. On the opposite side, smaller values (<5.4º) indicate that the integrity of body cells may be compromised, leading to a reduction of life span (Selberg & Selberg, 2002). According to this, a higher PhA suggests a more positive cellular health, integrity of cell membranes and better cellular function. Its values may vary between 5 and 7 in healthy people, and may even reach values near 9.5 in athletes. In the presence of disease, it is frequent to observe a smaller PhA,

since there may exist infection, inflammation, nutritional dysfunction or other specific parameters of disease itself, which compromise the PhA (Norman et al., 2012).

Bosy-Westphal et al. (2006) proposed reference values for PhA by age, sex, and body mass index (BMI) in both children/adolescents and adults/older adults. The authors observed that in youth, PhA increases with age, in both males and females and also with BMI. However, this would only be valid for children/adolescents, which means that from adulthood on, this relation becomes negative. The increment in PhA with age in the early years appears to be related to the increase in cell mass which is natural during growth and development (Bosy-Westphal et al., 2006). In addition, PhA seems to be higher in men compared to women, although this is not valid for children between 14 and 17 years old and after 70 years old. Regardless of sex and age, BMI explains 1% of the variance observed in PhA. In normal-weight and overweight individuals (BMIs up to 40 kg/m2), PhA and BMI are positively related. For BMIs higher than 40 km/m2, PhA decreases with BMI (Bosy-Westphal et al., 2006).

1.3.2. Implications in health

Evidence has shown that PhA is a useful, simple and inexpensive electrical biomarker for the prognosis of several health conditions, which will be discussed below.

Decreases in R and Xc may be caused by increased extracellular fluid or increased subcutaneous, intraabdominal and interstitial fat, which reduce cell wall and tissue permeability (Ackmann & Seitz, 1984; Baumgartner et al., 1988). Consequently, this affects the PhA, making it a great predictor of hydration status and % of body fat (BF). Specifically, PhA for the trunk may be used for the index of %BF and, regarding body segments, predict index adipose tissue distribution. Changes in fat mass (FM) and FFM and distribution of body water are likely to affect this relation between PhA and %BF. PhAs for the trunk, leg and whole body

are negatively correlated with %BF and positively correlated with FFM (Baumgartner et al., 1988).

Malnutrition results in unintentional weight loss, including lean mass, which may accelerate the progression of sarcopenia, which is very common in older adults (Janssen, Shepard, Katzmarzyk, & Roubenoff, 2004). During the aging process, malnutrition and/or sarcopenia, as well as frailty and cardiovascular disease (CVD) can be predicted through the measurement of the PhA (Basile et al., 2014; Janssen et al., 2004; Norman et al., 2012; Saad et al., 2018; Tomeleri et al., 2018).

Low PhA values are highly associated with mortality, disease progression, postoperative complications, chronic-degenerative and neoplastic diseases (Norman et al., 2012). Compared to normal individuals, PhA is significantly lower in people with osteoporosis, showing that it may be a risk factor and predictor for this condition, irrespective of age and sex (Tanaka et al., 2018).

PhA has also been shown to be correlated with diverse types of cancer, especially at advanced stages. Increasing PhA values seems to be crucial for the improvement in health status and higher survival rates and outcomes in cancer patients (Grundmann et al., 2015; Hui et al., 2014). In this sequence, several investigations found that PhA may be a strong predictor of advanced non-small cell lung cancer (Gupta et al., 2009), advanced head-and-neck cancer (Wladysiuk et al., 2016), advanced pancreatic cancer (Gupta et al., 2004) and advanced colorectal cancer (Gupta et al., 2008).

It is also useful in the prognosis of liver cirrhosis, since these specific patients usually present PhA values lower than 4.9°, which are associated with worse metabolic and nutritional status, and with disease progression profiles (Belarmino et al., 2017; Ruiz-Margain et al., 2015).

Lastly, PhA may also be used as a predictor of malnutrition in hemodialysis patients, which can be helpful in detecting and preventing malnutrition, allowing for the enhancement of quality of life of these patients (Rimsevicius et al., 2016).

When working with patients with diseases it is important to follow them throughout therapy and record their PhAs individually, so the prediction over time can be more accurate (Grundmann et al., 2015).

1.4. Impedance (Z), resistance (R) and reactance (Xc)

Typically, BIA uses a method which provides the values of R, Xc, Z and PhA. Z, measured in ohms, represents a complex value, which combines the two types of resistance that the body offers to an electrical current, which are capacitive resistance, known as Xc, and resistive resistance, simply called R (Kyle et al., 2004; Lukaski et al., 2017). Z is calculated by the square root of the sum of the squares of R and Xc (Z2 = R2 + Xc2), as illustrated in Figure 2 (Baumgartner et al., 1988).

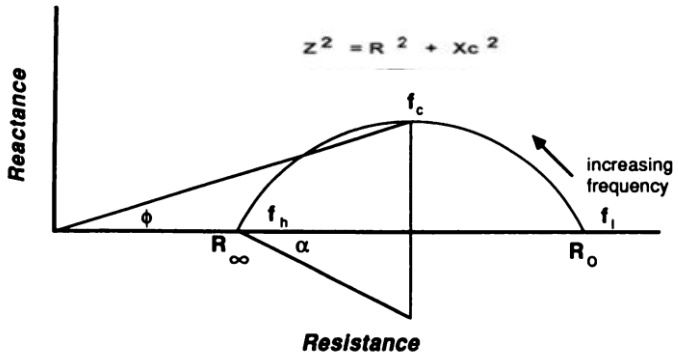

Figure 2: *Impedance plot illustrating the relationships between resistance (R), reactance (Xc), and phase angle (º) (Baumgartner, Chumlea, & Roche, 1988)*

Xc arises from cell membranes and represents the capacitive component of tissues, creating a lag in the current behind the voltage, which causes a phase shift, also called PhA.

On the other side, R arises from extra- and intracellular fluid, representing the opposition of a biological conductor to the flow of an alternating electric current (Baumgartner et al., 1988; Kyle et al., 2004; Lukaski et al., 2017).

R is constituted by the resistance components, such as water and electrolytes which can be found in tissues and fluids (Baumgartner et al., 1988; Kyle et al., 2004; Lukaski et al., 2017). According to Ohm`s law, the resistance of a substance is proportional to the voltage drop by the current which passes through it (Kotler, Burastero, Wang, & Pierson, 1996). Xc and R are related to each other, reflecting different electrical properties of tissues that may be affected in various ways by disease and nutritional or hydration status (Kyle et al., 2004).

Through the placement of low-Z electrodes, it is possible to measure Z and the delay between voltage and the electrical current in the tissue and cell membrane, which is expressed by the value of the PhA (Lukaski et al., 2017). PhA is negatively associated with R and positively with Xc (Baumgartner et al., 1988).

1.5. Total body water, extracellular water and intracellular water

Water is the largest chemical compound of the average human body, representing 60% and 55% of total body mass, for men and women, respectively (Martinoli et al., 2003). The total body water (TBW) compartment is the sum of the extracellular water (ECW) compartment (45% of TBW) and intracellular water (ICW) compartment (55% of TBW), all measured in liters (L) (Malbrain et al., 2014; Martinoli et al., 2003). ICW can be defined as the body water that exists inside the cell membrane, as ECW exists outside the cell membrane. ECW may be interstitial, lymphatic, trans-cellular fluid or blood (Malbrain et al., 2014).

BIA is considered to be an ideal method for the measurement of body water compartments (Thomas, Cornish, & Ward, 1992). Usually, the applied current used to access this compartments is 50 kHz, which allows to go through the ECW and ICW, and therefore

predict TBW (Ward et al., 2006). Studies show that SF-BIA and BIS significantly overestimate TBW in healthy individuals, which does not happen when using MF-BIA (Martinoli et al., 2003). Therefore, MF-BIA seems to be a more accurate method to determine TBW and to be more sensitive to changes in water compartments, even if body weight remains the same (Kyle et al., 2004; Martinoli et al., 2003).

Intracellular and extracellular body fluid status are of great importance in both healthy and diseased individuals (Malbrain et al., 2014). ECW and the ECW/ICW ratio are increased in the presence of edema, malnutrition and/or disease, such as heart failure, liver cirrhosis and chronic renal failure, which seems to be due to a shift from intra to extracellular space (Kyle et al., 2004; Malbrain et al., 2014). Changes in ICW are associated with changes in the metabolic and nutritional status of the organism. In the advanced state of some diseases, such as cancer and aids, there is a significant decrease of ICW, which reflects the loss of BCM (C. P. Earthman et al., 2000; Yoon, Grundmann, Williams, Gordan, & George, 2018).

Consequently, this affects the ECW/ICW ratio, which has shown to provide important information about hydration and BCM. Authors also believe that this ratio may be determinant for the PhA variation (Gonzalez et al., 2016), given the influence body water compartments and electrolytes have in resistivity (Kyle et al., 2004). Authors suggest that a low PhA value exists concurrently with a lower Xc due to a less amount of cell membranes and a smaller cell size, which is related with less ICW and more ECW (Foster & Lukaski, 1996; Guo, Chumlea, & Cockram, 1996; Malbrain et al., 2014). So, individuals with higher values of PhA usually have a lower ECW/ICW ratio (Buffa, Saragat, Cabras, Rinaldi, & Marini, 2013), due to higher BCM and therefore, lean mass (Buffa et al., 2013; Chertow et al., 1995). With aging, PhA tends to decrease as a consequence of smaller BCM followed by increases in the ECW compartment, which leads to a higher ECW/ICW ratio (Silva et al., 2005). The normal values for the ECW/ICW ratio are considered to be less than 1 (Malbrain et al., 2014).

1.6. Body cell mass (BCM)

Among the body compartments measured by BIA, there is also BCM, which is the protein rich compartment affected by catabolic states (Kyle et al., 2004). It is essentially the FFM with no bone mineral mass and extracellular water (Oliveira et al., 2010). BCM is considered one of the most metabolically active compartments in the body since it comprises all the cells which are capable of oxidizing substrates to obtain or convert energy (Moore & Boyden, 1963; Oliveira et al., 2010). It seems to be a reference marker for "energy exchange, work performance, and mitotic potential" (Moore & Boyden, 1963).

When the integrity of body cells is compromised, the capacitance drops, which directly affects PhA, causing its decrease (Selberg & Selberg, 2002). Thus, BCM, Xc, and PhA are related and seem to be useful biomarkers for nutritional status, specifically in patients with some kind of disease (Oliveira et al., 2010).

All body cells contain potassium and it appears that the concentrations of intracellular potassium are very similar between all cellular tissues. Therefore, the determination of the amount of potassium in the body may be the gold standard method to estimate the total weight of the BCM. By measuring the plasma potassium concentration and the extracellular fluid it is possible to calculate the total intracellular potassium (total exchangeable potassium - total extracellular potassium) (Moore & Boyden, 1963).

A different method would be measuring intracellular water, however it is not as accurate because this compartment may suffer from changes in the extracellular water (Moore & Boyden, 1963). BCM may also be determined through predictive equations based on BIA (Kotler et al., 1996). The equations used to measure BCM require the inclusion of H, otherwise it will not provide an accurate result. In patients with large alterations of body volume or hydration status, the use of BIA to measure BCM is not valid (Kyle et al., 2004).

2. Muscle

2.1. Skeletal Muscle Structure

Movement is only possible with an arrangement between skeletal muscles and bones, which are both connected by joints (Haff, Triplett, National, & Conditioning, 2016). Muscle contains muscle tissue, connective tissue, nerves and blood vessels (Haff et al., 2016). The muscle core is where muscle strength (MS) is produced and fascia and tendons are located in the edges to connect the muscle with the bone (Rasch, 1989). Muscle cells or muscle fibers comprise the contractile elements of the muscle (Drews & Wilmore, 2000; Pedro Mil Homens, 2005), such as myosin and actin which connect through cross-bridges and therefore generate MS (Nicpon-Marieb, 1992). The amount of strength created in the muscle depends on the amount of cross-bridges, the frequency of stimulation of the fiber and the accumulation of calcium inside the fiber (Pedro Mil Homens, 2005). Muscles are also composed by elastic components which allow them to have the ability of deforming and returning to their original shape (Rasch, 1989).

2.1.1. Types of muscle fibers

Muscle needs to adapt its capacities to different demands for movement and this seems to be guaranteed by the existence of different muscle fiber types according to twitch time and histochemical content: slow-twitch fibers (also known as red or type 1 fibers) and fast-twitch fibers (white or type 2 fibers), which can be subdivided in type 2A and 2X (Haff et al., 2016; Nicpon-Marieb, 1992).

Slow-twitch fibers are typically thin cells which are supplied by smaller motor neurons with smaller excitability thresholds and have, in their essence, an oxidative metabolism (Nicpon-Marieb, 1992; Powers & Howley, 1997). Thus, these fibers produce less tension and with a smaller intensity but are able to maintain it for longer periods of time, since they are

more resistant to fatigue (Bandy, Lovelace-Chandler, & McKitrick-Bandy, 1990; Morris, 1969). Fast-twitch fibers, namely type 2X are the opposite – they are inefficient, fatigable and have a glycolytic metabolism, so that they are primarily used in rapid force development (Haff et al., 2016). They are supplied by bigger size motor neurons and develop higher, faster, and more vigorous tensions but only for a short time period (Bandy et al., 1990; Morris, 1969; Pedro Mil Homens, 2005). Type 2A fibers comprise characteristics of both types (type 1 and type 2X), they are fast and intense and have a great resistance to fatigue (Powers & Howley, 1997).

2.1.2. Types of muscular action

Muscle contraction does not always imply the shortening of the muscle, it depends on the amount of strength produced and the magnitude of resistance offered by external forces (Nicpon-Marieb, 1992; Rasch, 1989). Three types of muscular action may be distinguished: dynamic (concentric or eccentric) or static (isometric) (Powers & Howley, 1997). Concentric muscle action is responsible for movement and acceleration and occurs when the tension produced by the muscle overcomes the external resistance, which leads to a shortening of the muscle (Karpovich & Sinning, 1971; Powers & Howley, 1997). By contrast, eccentric muscle action is important to cushion and stop movements and occurs when the muscle tension produced results in the elongation of the muscle (Karpovich & Sinning, 1971; Pedro Mil Homens, 2005; Powers & Howley, 1997). Finally, isometric muscle action happens when both tensions (internal and external) are equal, maintaining the length of the muscle (Rasch, 1989) and is crucial to fix and stabilise joints (Pedro Mil Homens, 2005).

2.2. Neuromuscular function

The central nervous system controls the activity of muscles and allows them to voluntarily contract, providing the necessary stimulus for the locomotor system (Pedro Mil Homens, 2005). In the next chapter, central nervous factors will be discussed, namely intra and intermuscular coordination, peripheral nervous factors and articular receptors.

2.2.1. Central Nervous Factors

Intramuscular Coordination

Motor neurons send impulses or action potentials that activate all its fibers, leading them to produce force. Muscles that require more precision have normally less muscle fibers for each motor neuron (Haff et al., 2016).

In this sequence, intramuscular coordination occurs when the central command, which produces movement, sends a certain amount of stimulus up to the group of motor units that form each muscle (Pedro Mil Homens, 2005). This process directly depends on two important mechanisms that help regulate the intensity of the contraction: the number of motor units recruited and the frequency of their activation (Nicpon-Marieb, 1992; Pedro Mil Homens, 2005). Concerning the first mechanism, the more motor units that are recruited, the greater the force of muscle contraction (Nicpon-Marieb, 1992). Henneman`s size principal sustains that motor units are recruited for crescent order according to their capacity to produce strength (Henneman, Somjen, & Carpenter, 1965).

Respecting the frequency of the activation of motor units, the higher the frequency of stimulus, the greater the intensity of fibers contraction (Nicpon-Marieb, 1992; Pedro Mil Homens, 2005). Tetanic contractions happen when a muscle is stimulated rapidly and repeatedly, allowing it to maintain the contraction for an uncertain duration (Nicpon-Marieb, 1992). The strength produced in a muscular contraction may increase through the increment in

both the number of motor units recruited and the frequency of activation of each motor neuron (Pedro Mil Homens, 2005).

Intermuscular Coordination

Intermuscular coordination relies on the coordination between all the muscles in the human body, which hold different structural and functional characteristics (i.e. agonist and antagonist muscles and fixator and neutralizer muscles) (Pedro Mil Homens, 2005; Rasch, 1989). Agonist muscles are the muscles that may potentially produce the articular movement and antagonist muscles are the natural opponents of agonist muscles (Rasch, 1989). The coordination between these two muscles is extremely important to increase the precision of movement. Fixators produce static strength and help fixating and stabilizing the origins of agonist and antagonist muscles. Neutralizer muscles are important to reduce the odds of developing a muscle injury, since they annul or reduce undesirable actions that may exist in the segment moved by a certain agonist muscle (Pedro Mil Homens, 2005; Rasch, 1989).

2.2.2. Peripheral Nervous Factors

Peripheral nervous factors are related to the processes of sensory innervation of the muscle and the influences deriving from the sensory receptors, which may lead to reflex responses. The proprioceptive receptors involved in this process are mechanic receptors, muscle spindles and Golgi tendon organs (GTO) (Pedro Mil Homens, 2005). Muscle spindles are located inside the muscle and are sensitive to the muscle stretching and length, informing about the speed and level at which the stretch is performed (Powers & Howley, 1997).

GTO are located inside the tendons and have a protective role in high intensity contractions, as they are connected to sensitive fibers which provide information about the level of contraction produced by the muscle (Haff et al., 2016; Powers & Howley, 1997).

2.2.3. Articular receptors

Articular receptors are located in the synovial membrane, the articular capsule and ligaments and provide information related to pain, joint positions, speed and amplitude of the movement. These receptors are only activated when the joints are in movement, given that their activity increases with the increment in the speed of the movement. They contribute to the reflex inhibition of antagonist muscles and the facilitation of agonist muscles, which justifies their importance in the prevention of injuries (Pedro Mil Homens, 2005).

2.3. Muscle Strength (MS)

2.3.1. Definition

The definition of strength and the way this term is used have been inconsistent and not consensual. However, it has been widely accepted that strength is the capacity to exert force at any given velocity (Haff et al., 2016).

Kroemer (1970) suggests that strength should be defined as "the maximal force muscles can exert isometrically in a single voluntary effort", considering that the muscular capacity to apply force is under static conditions. Therefore, strength may be referred as an impulse over a given time, force or torque alone if the effort, static or dynamic, is applied instantaneously (Kroemer, 1970). Although there are numerous definitions created by different authors addressing MS, the concept of strength does not provide useful information without referring which type of strength components is being considered (Pedro Mil Homens, 2005).

2.3.2. Strength Manifestations

The different strength manifestations which can be recognized are: maximal strength, speed strength, which includes rate of force development (or explosive strength) and muscle power, reactive strength, and strength endurance. For the purpose of this study, the maximal

strength for both lower and upper limb was measured. Thus, only maximal strength manifestation will be described below.

Maximal strength represents the upper limit of the ability to produce force and is manifested as the highest value of strength that can be produced in one contraction (Taber, Bellon, Abbott, & Bingham, 2016). It is considered to be the major form of strength since it is the component which relies more on muscle mass, influencing all the other expressions of strength. This type of strength should be optimally measured in isometric conditions, although it can still be expressed in a concentric or eccentric way (Pedro Mil Homens, 2005).

Functionally, maximal strength varies a lot in accordance with the muscular action and isometric action seems to be a special form of concentric action in which the speed of the movement equals zero. Sometimes there may exist a strength deficit, which is the difference between the maximal eccentric strength and the maximal concentric strength. The strength deficit indicates the capability of nervous activation (Pedro Mil Homens, 2005). Within maximal strength, absolute and relative strength should also be considered. Absolute strength is the highest value of strength regardless of the person`s body mass and relative strength is the highest value of strength per each unit of body mass (Pedro Mil Homens, 2005).

2.3.3. Measurement of maximal strength

There are several ways to assess MS depending on the main objective of the test. For the purpose of this study, which was testing maximal strength, it was used a maximum voluntary isometric contraction (MVIC) testing with fixed-load cells (Amundsen, 1990). This is a secure and objective method, which allows testing both strong and weak muscles in the most accurate and reliable way (Amundsen, 1990; Meldrum, Cahalane, Keogan, & Hardiman, 2003). This can be performed in all the major muscle groups of the body, but only one or two muscle groups can be tested at the same time (Amundsen, 1990). The force applied by the

individual performing the test creates a voltage, which is converted by computer into Newtons or kilograms (Meldrum et al., 2003). Maximum values are generally seen within 3–4 seconds and standardized verbal encouragement should be given during each trial (Meldrum et al., 2003).

This method allows the observation of different components of maximal muscle contraction: reaction time, rise time, peak force, and fatigue (Amundsen, 1990). Reaction time is the delay time that occurs between the time when stimulus is recognized until the activation of the contractile mechanism and lasts less than 0.4 seconds in younger healthy participants (Amundsen, 1990). Rise time, represented by the rate of recruitment of motor neurons, which is influenced by the muscle fiber types recruited, lasts normally 0.3 seconds and is highly influenced by motivation and learning (Amundsen, 1990). Peak force is the index of maximal voluntary MS and depends on the ability of the central nervous system to send enough impulses at high frequencies to synchronize as many motor neurons as possible (Amundsen, 1990). It involves factors such as motivation, experience, muscle mass and muscle fiber type (Amundsen, 1990). At last, fatigue stands for the rate of the decrease in force and represents an index of absolute endurance for the single muscle contraction (Amundsen, 1990).

3. Association between phase angle (PhA) and muscle strength (MS)

Low skeletal muscle mass and strength are associated with shorter survival rates. It has been observed by some authors that, independently of factors such as age, PhA is positively correlated with muscle function, which includes MS, capability to perform daily activities, and muscle mass (Basile et al., 2014; Beberashvili et al., 2014; Norman et al., 2012; Norman et al., 2010). There are reports showing that higher values of PhA exist in parallel with higher MS values. These evidence suggest that PhA is a useful predictor of muscle dysfunction (Beberashvili et al., 2014), as low levels of PhA are highly related to a reduction in muscle

mass and strength (Basile et al., 2014). Previous studies have found that PhA had a strong association with muscle function parameters, particularly hand grip strength and knee extension (Norman et al., 2012; Norman et al., 2010). Also, a different study observed that after a period of detraining, both PhA and MS decreased in older women (Dos Santos et al., 2016). Because of the significant relation found between PhA and muscle function, PhA may be an important biomarker to detect situations of sarcopenia in older adults (Basile et al., 2014; Norman et al., 2012; Tomeleri et al., 2018). The optimal PhA cut-off value to detect sarcopenia seems to be $\leq 4.55^{o}$ (Kilic et al., 2017). Therefore, it seems evident that the leading role of exercise in improving general health is through the increase of muscle mass and strength, and therefore PhA.

4. Sedentary Behaviour (SB)

4.1. Background and Definition

According to Sedentary Behaviour Research (2012), SB is defined as any waking behaviour characterized by an energy expenditure ranging from 1.0–1.5 METs (metabolic equivalents or multiples of the basal metabolic rate) in a sitting or reclining position. SBs are characterized by relatively low energy expenditure, with lack or absence of muscular contraction (Sedentary Behaviour Research, 2012).

The evolution of industrialization and technology have been leading people to spend more time in SBs, essentially sitting, causing adverse outcomes, such as the significant decrease of movement performed by the large skeletal muscles in the legs, back and trunk which are required for upright movement (Hamilton et al., 2007; Hill et al., 2003; Lanningham-Foster et al., 2003).

It is well known that most SBs involve sitting for extended periods (Katzmarzyk et al., 2009). Although the total time spent in SB is important, the way in which it is accumulated

seems to highly contribute to the negative effects on health (Healy, Dunstan, et al., 2008). Generally, there are two different patterns of SB: continuous, in which people accumulate prolonged and uninterrupted periods of SB; and discontinuous, with the interruption of SB, even if the breaks are short (Judice, Silva, Santos, Baptista, & Sardinha, 2015; Tremblay et al., 2017).

4.2. Prevalence

Bauman et al (2018) objectively measured time spent in SB and found that adults spend an average 8.2 h/d and older adults spend more time in SB compared to adults overall (A. E. Bauman et al., 2018). This goes according to the findings of Harvey et al (2013), who objectively measured SB in older adults, determining that about 67% were sedentary for more than 8.5h in their waking day (Harvey, Chastin, & Skelton, 2013). A more recent systematic review concluded that older adults spent a total sitting time of ~9.4 h, recorded objectively by accelerometers (Harvey, Chastin, & Skelton, 2015).

An epidemiologic study using the International Physical Activity Questionnaire (IPAQ) to measure the prevalence of sitting time in 20 countries, provides evidence that, generally, people spend around 346.2 minutes per day in a seated position (interquartile range from 180 to 480 minutes), which is equal to about 5 to 6 hours a day (A. Bauman et al., 2011).

Bauman et al. (2011) found that, between 20 countries (study mentioned above), Portugal is one of the countries reporting the lowest amount of sitting, having about 50% of the total sample representing the first quintile (0-179 minutes a day) (A. Bauman et al., 2011). A study from 2018 collected data from a representative sample of the noninstitutionalized population of Portugal (10 to 102 years old) and observed that older adults, in comparison to other age groups, spent larger amounts of sedentary time considering bouts of ≥ 30 min. Of

total sedentary time, 33% was spent by older women and 39% by older men (Santos et al., 2018).

4.3. Impact of Sedentary Behaviour on health outcomes

It is well known that the adoption of SBs is highly associated with health risks (van Uffelen et al., 2010). Thus, lifestyle modifications are of great importance and may effectively attenuate the deleterious effects of spending prolonged time in these behaviours (Dempsey, Owen, Biddle, & Dunstan, 2014). Despite all the existing research, more investigation is needed to extend the findings to all populations and predict how much SB impact health (Craft et al., 2012).A review of the harms that emerge as a consequence of SB will be presented bellow according to the study category: observational studies, experimental studies and systematic reviews and meta-analysis.

4.3.1. Observational Studies

Authors who conducted observational studies reported that SB can dangerously increase waist circumference, total cholesterol, LDL cholesterol and triglycerides, and also the risk of insulin resistance, metabolic syndrome and mortality from all causes (Healy, Wijndaele, et al., 2008; Katzmarzyk et al., 2009; van der Berg et al., 2016). Each increase of one h/d in time spent in SB seems to be associated with an increased risk of mortality from all causes, except cancer (Wijndaele et al., 2011). Also, women who spend less than half of their daily time sitting have a reduction in the risk of all-cause mortality compared with those who spend more than half of their day sitting (Weller & Corey, 1998). A recent study (2018) used a sample of regularly active adults and implemented a short-term reduction in physical activity, specifically in the amount of daily steps, with increased sedentary time. They found deleterious effects, such as lower whole-body insulin sensitivity, muscle insulin sensitivity index,

cardiorespiratory fitness, limb lean mass and higher total body fat, liver fat and LDL-cholesterol (Bowden Davies et al., 2018).

A study on older adults found that for each hour increment in sedentary time, there is a 48% increment in the probability of being abdominally obese (Judice, Silva, & Sardinha, 2015). When considering breaks, for each additional hourly break in sedentary time, there is a 7% decrease in the odds for abdominal obesity in older women (Judice, Silva, Santos, et al., 2015). Also concerning older adults, regardless of physical activity, each 1h increment in sitting time seems to lead to an increase of 33% in the risk of sarcopenia. TV viewing time, specifically, is associated with reduced total and leg muscle mass (Gianoudis, Bailey, & Daly, 2015).

4.3.2. Experimental Studies

Comparing continuous sitting to standing in desk-based workers, authors found that standing helps attenuate blood glucose by 43% and increases substantially energy expenditure. This suggests that avoiding SB could contribute to the enhancement of insulin sensitivity and the reduction of the risk of cardiometabolic diseases (Buckley, Mellor, Morris, & Joseph, 2014). In similar intervention, sitting time was compared with short bouts of light and moderate intensity walking in overweight/obese adults (Dunstan et al., 2012) and postmenopausal women (Henson et al., 2016). The results showed that interrupting SB helped to decrease postprandial glucose and insulin levels, improve glucose metabolism and reduce cardiovascular risk (Dunstan et al., 2012; Henson et al., 2016). A Randomized Controlled Trial (RCT) used a sample of sedentary men and women and found that adults who engage in regular, long bouts of walking significantly enhanced feelings of vigour and activity, decreased SB, reduced percent body fat, and had more energy along with less tension and anxiety (Osei-Tutu & Campagna, 2005).

Judice et al. (2016) (Judice, Hamilton, Sardinha, Zderic, & Silva, 2016) quantified the metabolic/energy cost (MEC) (5 min condition) of three conditions (sitting, standing, sit/stand transition) using standing desks and related that for every 10 sit/stand transitions (sit to stand followed by a stand to sit movement), the metabolic rate is increased modestly (~3.2 kcals) but significantly above sitting. They found that working at a standing desk may produce a significant rise in energy expenditure compared with sitting.

4.3.3. Systematic Reviews and Meta-analysis

Wilmot et al. (2012) observed that, independently of physical activity, SB was associated with a 112% increase in the relative risk of diabetes, 147% increase in the risk of cardiovascular disease, 90% increase in the risk of cardiovascular mortality and 49% increase in the risk of all-cause mortality (Wilmot et al., 2012). Different systematic reviews stated similar findings - regardless of physical activity, SB appears to be a distinct risk factor for multiple health outcomes, such as premature mortality, specifically all-cause and CVD-related mortality, site-specific cancer, relative risk of type 2 diabetes, mental disorders, hypertension, and adiposity impairments (obesity or overweight) (Groeneveld, Proper, van der Beek, Hildebrandt, & van Mechelen, 2010; Thorp, Owen, Neuhaus, & Dunstan, 2011). Chau et al. (2013) observed in their meta-analysis that every hour of daily sitting time was associated with a 2% increase in all-cause mortality risk, which increased to 5% for those sitting >7 hours/day, although this association was attenuated in the presence of MVPA (Chau et al., 2013).

A more recent systematic review and meta-analysis concluded that, irrespective of physical activity, total sitting and TV viewing are positively associated with an increased risk for all-cause and CVD mortality (stronger for volumes greater than 6-8 h/day of total sitting and 3-4 h/day of TV viewing), and likelihood of type 2 diabetes (Patterson et al., 2018).

Between 43 studies (17 case-control and 13 prospective studies), 17 examined the associations between occupational sitting and cancer and found positive associations with risk of breast cancer, ovarian cancer and colon or rectal cancer (van Uffelen et al., 2010).

5. Physical (In)Activity

5.1. Definition and Recommendations

Caspersen et al (1985) defined physical activity as any bodily movement produced by skeletal muscles above resting levels, which may be measured in kilocalories and expressed as a rate (kcal per unit time) that varies continuously from low to high. In daily life it is possible to find physical activity in diverse activities, such as occupational activities, sports, conditioning, household tasks or others (Caspersen, Powell, & Christenson, 1985).

American College of Sports Medicine (ACSM) provided a recommendation for physical activity, which should be the weekly accumulation of at least 150 minutes of at least moderated physical activity, or 75 minutes of vigorous physical activity, or a combination of both (American College of Sports, Riebe, Ehrman, Liguori, & Magal, 2018).

In Portugal, there is a prevalence of 70% of adults between 18-64 years old attaining the recommendations for physical activity. About 35% of people older than 64 years reaches the recommendations of 30 minutes of physical activity, with a prevalence of 46% for men and 29% for women (Baptista et al., 2012).

The engagement in physical activity regularly is highly important to reduce the risk of premature death and to prevent, primarily and secondarily, several chronic diseases, such as diabetes mellitus, hypertension, obesity, osteoporosis, cancer, depression and all-cause and cardiovascular-related death. People who surpass the recommendations for physical activity are more likely to achieve further gains in their health status (Warburton, Nicol, & Bredin, 2006). This is also valid in overweight and obese individuals – those who are inactive and unfit

have higher rates of disease and premature death compared to those who are active and fit (Blair & Brodney, 1999). However, it seems that people who are inactive and become physically active have the greatest improvements in health. Sometimes, increasing physical activity levels may enhance indicators of health status without inducing changes in fitness performance, which is very typical in older people (Warburton et al., 2006).

Considering physical inactivity, accordingly to the WHO, in 2008, around 31% of adults (15 years or older) were insufficiently active (28% men and 34% women). As a consequence of inactivity, about 3,2 million deaths occur each year (WHO, 2008). In 2009, the WHO identified physical inactivity as the fourth leading risk factor for global mortality (WHO, 2009, 2010). More recently, in 2011, the overall prevalence of physical inactivity was 21.4% and 17.4% after weighting for the total population of each country (Dumith, Hallal, Reis, & Kohl, 2011). One out of five adults around the world was physically inactive and it tends to increase with age. In addition, women are more likely to be inactive than men in most countries (80%) (Dumith et al., 2011).

5.2. Confusion between physical inactivity and sedentary

The term *'sedentary'* can be misleadingly described as nonparticipation in moderate-to-vigorous activity (MVPA), which means it would represent the lower end of the physical activity continuance (Dempsey et al., 2014). Therefore, it is suggested by Sedentary Behaviour Research (2012) that authors use the term "inactive" to describe people who do not accumulate sufficient amounts of MVPA (i.e., not meeting specified physical activity guidelines).

Ekblom-Bak et al. (2010) showed how MVPA, light-intensity activity, and sedentary time are related and manifested in real life. A large part of the adult populations do not engage in MVPA and spend much of their waking hours sitting, which means they are both "sedentary and inactive" (Ekblom-Bak, Ekblom, & Hellenius, 2010). However, people can be "sedentary

and active": for example people who work all day in a seated position but exercise in some part of the day (Healy, Wijndaele, et al., 2008). A less common behaviour pattern is being "peripatetic and inactive", in which the individual is not participating in MVPA, but sits very little time throughout the day. This seems to be the most realistic strategy to adopt, at least initially, given the fact that most of populations are highly sedentary. For optimal health outcomes, the best condition would be being "peripatetic and active", having reduced levels of sedentary time, specially sitting, and sufficient amounts of MVPA (Ekblom-Bak et al., 2010).

6. Detraining

6.1. Definition and general effects on health

Detraining may be defined as the partial or complete loss of training-induced physiological, anatomical adaptations and athletic performance, in response to an insufficient or inexistent training stimulus (Fleck, 1994; Mujika & Padilla, 2000). The consequences of detraining rely on specific characteristics, such as its duration, frequency and intensity (Fleck, 1994; Mujika & Padilla, 2000). Although there are well-known benefits from exercise training, it is very common for older adults to voluntary engage in training cessation, whether is due to the participation in extended holidays, volunteering, or family commitments. Also, dependent older adults with advanced morbidity may be forced to be inactive (Henwood & Taaffe, 2008). Besides the consequences observed on physiological and physique markers (e.g. body composition, lipids, insulin sensitivity, bioelectric parameters, cardiometabolic markers) (Bowden Davies et al., 2018), after a period of detraining, it is possible to observe losses in the endurance capabilities, such as $VO_{2\ max}$, due to different mechanisms (e.g reduced stroke volume, cardiac output, blood volume, cardiac hypertrophy and contractility, etc.), and losses in the skeletal muscle, which results in a decrease in MS and muscle power (Fleck, 1994).

6.2. Effects of detraining on phase angle (PhA) and muscle strength (MS)

There is little evidence concerning the effects of detraining on PhA. It is known that high values for PhA are related with high physical activity levels in non-institutionalized older people (Norman et al., 2012). So far, only one study focus on these issue and authors have found that an interruption in the training routine may cause a significant decline in the PhA, which is greater than the gains obtained with previous training, and which can only be recovered partially with retraining (Dos Santos et al., 2016).

Concerning MS, evidence shows that detraining is associated with a loss in both muscle mass and strength, and its magnitude depends on the duration of this interruption. Interventions performed with older people observed that after long periods of detraining, there was a decrease in maximal strength and in muscle mass, however, these values did not regress to the baseline values previous to the participation in the training program (Tokmakidis, Kalapotharakos, Smilios, & Parlavantzas, 2009).

Some authors studied the effects of resistance training and detraining in muscle quality and the evidence is contradictory. Ivey et al. (2000) found that strength training increases muscle quality both in men and women, regardless of age. However young women may experience a larger gain in muscle quality compared to men and other age groups. After the training program, there was a detraining period, and they found that all groups, with the exception of older women, were able to keep the positive effects induced by strength training for at least 31 weeks of detraining (Ivey et al., 2000).

A study observed the effects of detraining on MS and mass after a program of resistance training in older adults and found that a substantial portion of recently acquired MS can be retained following a short period of detraining. Also, they observed that there was a rapid strength gain after retraining. The preservation of strength after detraining seems to be caused by retention of neural adaptations, since the values of cross-sectional area of type 1 and II fibers

returned to pre-training values. This evidence suggests that older people have the same muscle adaptability as younger adults and a short period of the cessation of training will not have a deleterious effect on MS (Taaffe & Marcus, 1997).

Henwood & Taaffe (2008) were the first to "compare change in muscle function and physical performance following detraining and retraining in previously power- or strength-trained older adults". They observed that the muscle power decreased following 24 weeks detraining but this was regained during the following 12 weeks of retraining, independently of change in movement velocity, suggesting that the component having the greatest impact on power loss during detraining was muscle power. The results show that a prolonged period of detraining resulted in a significant decrease in muscle function among older adults who previously engaged in muscle power or strength training. Also, these losses were reversed with 12 weeks retraining (Henwood & Taaffe, 2008).

7. Ageing

7.1. Definition and ageing process

The process of aging is different for every individuals, however there has been an agreement that the threshold of old age should be 65 years (Shepard, 1997).

Through aging, several changes occur on some of the major physiological and biological systems of the body. There is usually a reduction in the ability of adaptation and maintaining a state of homeostasis (Bales & Ritchie, 2002; Shepard, 1997). Concerning the cardiovascular system, there is an increase in risk factors associated with the development of CVD, such as metabolic syndrome, insulin resistance, dyslipidemia, higher fat deposition, hypertension and diabetes mellitus (Ravaglia et al., 2006; Saad et al., 2018). These contribute to the increased risk of CVD, which is one of the main causes of morbidity and mortality in older people (Saad et al., 2018). Also, dysfunction of connective and musculoskeletal tissues

start to occur - bones become weaker because of a reduction in the amount of bone tissue; chondroid tissues are affected as cartilage looses resilience; ligaments lose elasticity and, therefore, the ability to absorb shock loading and return to their original shape; and skeletal muscle becomes weaker and smaller (Freemont & Hoyland, 2007; Shepard, 1997). These changes may be explained by a decline in the efficiency of functional tissue elements, reduction of the synthesis capacity of differentiated cells and alteration in the levels of trophic hormones, growth factors and cytokines (Freemont & Hoyland, 2007). Difficulties may arise in vision, hearing and in the endocrine, gastrointestinal and renal systems. Brain also suffers from aging, showing its manifestations in memory, cognition and learning. In the respiratory system, there is a loss of elasticity in the pulmonary tissue, tidal volumes and capacity changes, as well as gas distribution (Shepard, 1997).

All of these age-related changes may be pathological and lead to a dependent state in which the older adult may have poor mobility, weakness, increased risk of falling, fractures or even diseases, such as osteoporosis or osteoarthritis (Freemont & Hoyland, 2007). Sedentary behaviour may be a modifiable risk factor that can improve quality of life of older adults and reduce the risk of disease and disability (Dogra et al., 2017).

7.2. Implications on phase angle (PhA)

Evidence suggests that age is a determinant factor in the PhA variation, and there is a negative correlation between the two, both in men and women. PhA is not only a body composition and nutritional state indicator, but also a marker for general function and health (Barbosa-Silva, Barros, Wang, Heymsfield, & Pierson, 2005).

PhA tends to decrease with aging as a consequence of the reduction of Xc and the increase of R, caused by the decline of body water proportions and body composition (increases FM and reduced FFM) (Norman et al., 2012). Therefore, it is a great predictor of reduced body

cell, reduced nutritional status (Slee, Birc, & Stokoe, 2015) and loss of lean mass (Norman et al., 2012). Lower values of PhA are related to a four-fold and three-fold odds of frailty in women and men, respectively. This is highly associated with mortality, independent of age and comorbidity (Wilhelm-Leen, Hall, Horwitz, & Chertow, 2014).

Sarcopenia is very common in older adults and may, in part, depend on the nutrition, specifically dietary protein intake. Malnutrition in aging exacerbates the progression of sarcopenia. However, to contradict this state, authors have shown that eating enough protein may help to preserve lean mass, and thus, contribute to the reduction in the normal progression of sarcopenia in older adults (Janssen et al., 2004). This state of malnutrition may be predicted through the measurement of the PhA, given its relation with the amount and quality of soft tissue, hydration status and therefore, nutritional status (Lukaski et al., 2017). In geriatric populations this seems to be crucial since it provides information on oxidative stress, inflammatory biomarkers and, therefore, sarcopenia (Basile et al., 2014; Norman et al., 2012; Tomeleri et al., 2018).

Also, in older adults, PhA correlates with systolic blood pressure, neck circumference and fat-free mass, suggesting that it has an independent association with global cardiovascular risk and, therefore, seems to be a useful marker for predicting CVD (Saad et al., 2018).

7.3. Implications on muscle strength (MS) and mass

The ageing process involves impairments in muscle function, such as muscle atrophy (reduction in muscle mass), weakness, injury and fatigue, which results in the decrease of absolute values of strength, power and endurance (Faulkner & Brooks, 1995; Powers & Howley, 1997; Shepard, 1997). All of these events contribute to the development of the age-related disease, sarcopenia, which has already been discussed above (Freemont & Hoyland, 2007; Lexell, Taylor, & Sjostrom, 1988).

Lexell et al (1988) argued that the mechanisms behind muscle atrophy can be both a loss of muscle fibers and/or a decrease in their size, which explains why elderly have less proportions of muscle compared with younger people. The reduction of muscle fiber number and the specific type of fiber has the greatest influence on the area and proportion of muscle loss, which usually begins as early as 25 years old, accelerating from then on. There is a progressive decline in neurogenic processes, which contribute to the atrophy of muscles. This is due to the decline in the capacity of neurons to innervate the muscle, leading to the loss of muscle fibers, which become deinervated (Lexell et al., 1988). The reduction in fibers size seems to be mostly observed in type 2 fibers. Different muscles in the human body appear to be differently affected by ageing, since they have distinct functions, structures and plasticity (Lexell et al., 1988).

Objectives and relevance of the study

As far we know, only one study studied the effects of detraining in both PhA and MS (Dos Santos et al., 2016). Additionally, most studies assessing the effects of detraining are performed with adult populations (up to 64 years old). Hence, considering the lack of studies approaching the effects of reducing the levels of physical activity in trained populations, namely older adults, this study emerged because of the need to understand the magnitude of the effects of short-term detraining in this particular population, specifically regarding PhA and MS.

The main objective of this study was to observe the effects of 2 weeks of detraining (i.e. refrain from exercise) on PhA and MS, in older adults. A secondary objective of this study was to observe if PhA and MS are related.

It is hypothesized that PhA and MS will decrease after 2 weeks of detraining. Additionally it is hypothesized that PhA and MS will be positively correlated.

Methodology

1. Recruitment Process

Participants were recruited between November 2017 and March 2018 to take part in an intervention study for older adults. Media advertisements and attendance to local exercise classes were used to recruit the participants within the region of Oeiras –Portugal. Interested participants carried out the enrolment process (Figure 3), starting with a recruitment session, where it was provided a thorough explanation of the intervention. In this presentation participants had access to the following information: main goals, details of the intervention in which they would engage, and requirements to be a part of the study in terms of schedule and time availability. At the end of the presentation participants filled in a questionnaire (Appendix A) to ascertain who met the inclusion criterion. Written informed consents (Appendix B) were obtained from all participants before and prior to any protocol-specific procedures.

In order to be a part of this intervention, the participants had to be aged between 65 and 90 years, physically active, and engage in structured exercise at least twice a week, for the past 6 months. People who had any type of severe limitation that would prevent them from practicing exercise, were excluded from the sample. Power and sample size calculations (G*Power 3.1.9.2) were based on an effect size of 0.78 for the glucose iAUC, while using the t-test for paired samples, a power of 0.80, and a significance of 0.05 (Hawari, Al-Shayji, Wilson, & Gill, 2016). The calculation yielded a sample size of 15 participants, while expecting a dropout rate of 10%. For the present dissertation a total of 14 participants were recruited and enrolled (Figure 3).

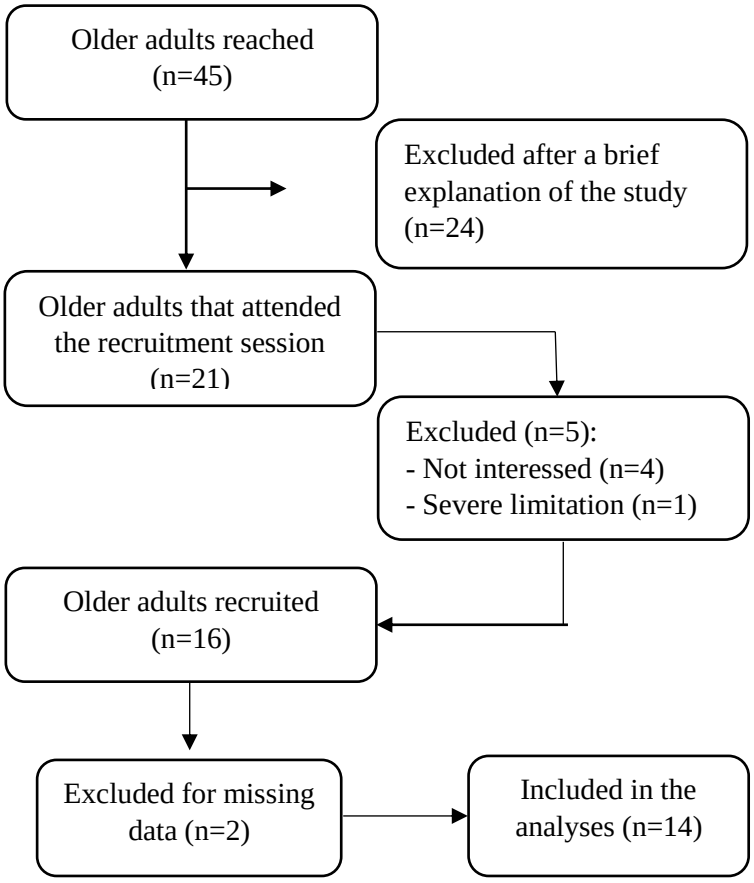

Figure 3: *Flow Chart*

2. Study Design

Participants were followed in a crossover experimental design. Physical fitness, anthropometric and body composition measurements were assessed in two separate days (Figure 4), at baseline and after 2 weeks of detraining. This intervention took place in *Faculdade de Motricidade Humana*, *Universidade de Lisboa*, and occurred over the course of 6 months. The present investigation was approved by the Ethical Committee of the faculty (approval number: 06/2019).

Three different dissertations with distinct outcomes – one for glycaemic control, other for metabolic flexibility and the last one for phase angle and muscle strength - were

accomplished performing the same intervention. However, the primary outcome of the intervention concerns changes in iAUC glucose.

In the first day of the assessments, each person received their schedule in the paper, which contained details about the location, time and the activities to complete.

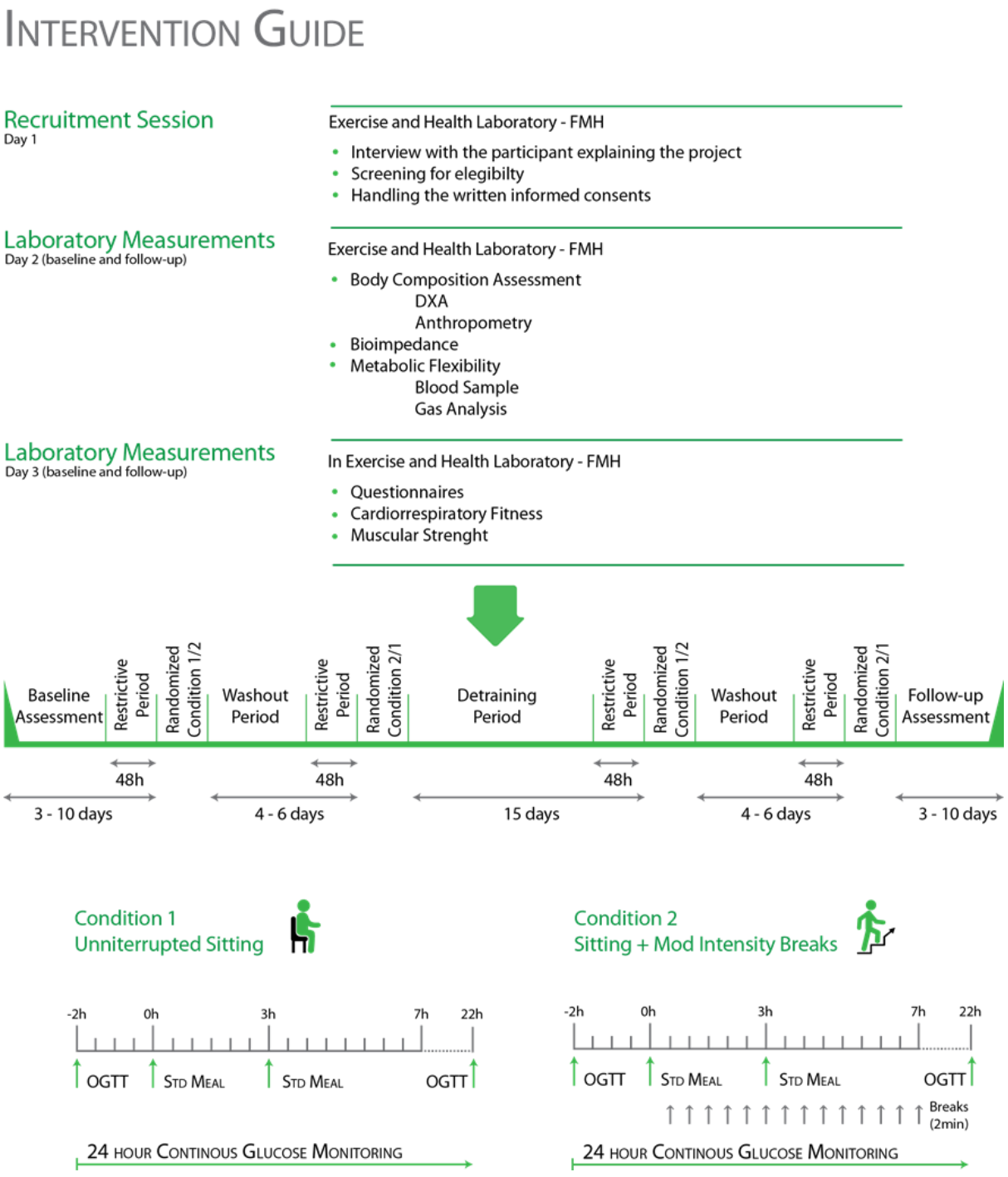

Figure 4: *Intervention Guide comprising all the assessments and protocols from the 3 studies included in the intervention*

3. Baseline and follow – up assessments

3.1. Anthropometric measures

Participants were weighed on an electronic scale without shoes wearing minimal clothing to the nearest 0.01 kg (Seca, Hamburg, Germany). Height was measured to the nearest 0.1 cm with a stadiometer (Seca, Hamburg, Germany), according to standardized procedures (Lohman, Roche, & Martorell, 1988).

3.2. Body composition measurements

Dual energy X-ray absorptiometry (DXA) (Hologic Explorer-W, fan-beam densitometer, software QDR for windows version 12.4, Waltham, USA) was used to estimate total FM, abdominal FM, FFM, appendicular lean mass (APM), bone mineral content (BMC) and body mass index (BMI). A whole-body scan was performed and the attenuation of X-rays pulsed between 70 and 140 kV synchronously with the line frequency for each pixel of the scanned image that was measured. Abdominal and gynoid body FM were measured through partial analyses of the DXA scan, based on regions of interest (ROIs) set by default on the DXA settings. Following the protocol for DXA described by the manufacturer, a phantom with six fields of acrylic and aluminum of varying thickness and known absorptive properties was scanned alongside each participant to serve as an external standard for the analyses of different tissue components. The same laboratory technician positioned the participants, performed the scans and executed the analyses according to the operator's manual using the standard analysis protocol. Based on ten participants, the coefficients of variation in our laboratory for FM and abdominal FM were 1.7% and 0.01%, respectively.

Following DXA, the participants did the BIA analysis (single frequency, 50 kHz ± 1%, NutriLAB, Akern) in order to determine the PhA, R, Xc and body water compartments (TBW and ECW directly measured and calculated ICW). The participants should be in a lying

position, with legs apart from each other and arms apart from the trunk, so that the medial surface of the limbs would not touch the rest of the body (Selberg & Selberg, 2002); 4 electrodes (2 in each limb) were placed on the hand and foot of the right side, with a distance of 5 cm between both.

3.3. Physical Fitness

3.3.1. Cardiorespiratory fitness

Cardiorespiratory fitness was determined using a modified Bruce protocol (Noonan & Dean, 2000) on a motorized treadmill to exhaustion (model Q-65, Quinton, Cardiac Science Corp; Bothell, WA, USA). Prior to the test, participants were familiarized with the protocol and with the Borg Rating of Perceived Exertion Scale (RPE) (Borg, 1982). At the end of each stage, they were requested to rate their perceived exertion using the Borgs scale .All graded exercise tests were monitored using a 12 lead electrocardiogram PC-based acquisition module by a certified cardiologist, and all data, including heart rate, were monitored and recorded using Omnia software (Modelo ECG). Inspired and expired gases were continuously analysed, breath-by-breath, through a portable gas analyzer (QUARK RMR, version 9.1, Cosmed, Rome, Italy). Participants exercised until at least two of the following test termination criteria were reached: (1) participants volitional fatigue; (2) respiratory exchange ratio reached 1.1 or higher; (3) participants reached predicted maximal heart rate; (4) oxygen uptake did not increase in spite of increasing workload (Milani, Lavie, Mehra, & Ventura, 2006; Pescatello & American College of Sports, 2014). The highest 20 seconds value for peak oxygen consumption (ml/kg/min) attained in the last minute was used in the analysis.

3.3.2. Muscle Strength

Muscle strength was assessed on both the lower and upper limbs, using the leg press machine and the bench press, respectively, under isometric conditions (Hyatt, Whitelaw, Bhat, Scott, & Maxwell, 1990; Lovell, Cuneo, & Gass, 2010). Before each testing, the participant was instructed to perform 5 minutes of warm-up, followed by a moment of familiarization with the movement techniques for both the upper and lower limbs. The assessment of the lower limbs was performed through an isometric test of horizontal *Leg Press* (S0409, HBP), in which the participant had his thigh flexed and the knee joint creating an angle of 110º. For the upper limbs, the assessment of maximal strength was performed through a test of isometric supine (*multipower, Technogym*), with the elbow flexed and the shoulder joint creating an abduction of 90º. In both testing, the participants completed 4 maximal voluntary repetitions with the duration of 3-4 seconds, with 1min and 30sec recovery between trials. After a countdown and while getting a verbal stimulus, the participants were instructed to produced maximal strength as fast as possible in every repetitions (Mil-Homens, Pezarat-Correia, & Mendonça, 2017). The software used to analyse the highest value recorded between all the maximal voluntary repetitions was the acqKnowledge 4.1 – BIOPAC Systems, Inc.

4. Detraining period

After baseline assessments, participants underwent a detraining period of 15 days, in which the objective was to significantly reduce the levels of physical activity (Figure 4). During this period, participants were instructed to refrain from structured and supervised exercise sessions (Esain, Gil, Bidaurrazaga-Letona, & Rodriguez-Larrad, 2018) at their local gym classes, and were also advised to reduce their daily levels of physical activity (e.g. avoid long walks). All physical activity performed by the participants during these 15 days period was monitored by accelerometers.

At the end of the detraining period, participants underwent the same assessments performed at baseline (anthropometric and body composition measures, cardiorespiratory fitness and muscle strength assessments).

5. Objective Measures of Sedentary Time and Physical Activity

Sedentary time and PA were assessed by accelerometry (ActiGraph, GT3X model, Fort Walton Beach, FL) before the detraining period, at baseline (for 1 week, including both week and weekend days), and during the 2-weeks of detraining, to allow comparisons between their physical activity in a free living daily life and during a detraining period. The accelerometer is a small device that measures the acceleration of normal human movements, ignoring high frequency vibrations associated with mechanical equipment. All participants were asked to wear the accelerometer on the right hip, close to the iliac crest. The devices were activated on the first day (in the morning) and data were recorded in 60 seconds epochs. Apart from accelerometer non-wear time (i.e., when it was removed during sleep and bathing activities), periods of at least 60 consecutive minutes of zero activity intensity counts were also considered as non-wear time (Colley, Connor Gorber, & Tremblay, 2010).

A valid day was defined as 600 minutes (10 hours) or more of monitored wear time, and all participants were instructed to wear the equipment during the detraining period (2-weeks). If the participants were unable to use it throughout the detraining period, they had to use the equipment at least 3 valid days (including one weekend day). The device activation, download, and processing were performed using the software Actilife (v.6.9.1). The cutoff values used to define the intensity of PA and therefore to quantify the mean time in each intensity (sedentary, light, moderate or vigorous) were as follows: sedentary: < 100 counts·min-1; light: 100-2019 counts·min-1; moderate: 2020-5998 counts·min-1 (corresponding to 3-5.9 METs); vigorous: ≥ 5999 counts·min-1 (corresponding to ≥ 6 METs)

(Troiano et al., 2008). There are no cutoffs for the sedentary-time using the three-axial information from this new generation Actigraph GT3X+ accelerometer; therefore we used the previous cutoffs based on the vertical-axis only.

6. Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics for Windows version 24.0 (SPSS Inc., IBM Company, Chicago IL, USA). The statistical significance used was $p < 0.05$ and a power of 0.80 for all variables and analysis.

Descriptive statistics (means ± standard deviation) were performed for all outcome measurements. The general linear model for repeated measures was performed with adjustment for the co-variable sex. The normality was assumed and the Greenhouse-Geisser test was used to read the significance between moments.

We used Adobe Illustrator CC 2018 to build the BIA vector (BIVA) which uses whole-body R and Xc values, normalized for standing H and the reference population (Piccoli et al., 1995a). Ellipses indicate sex-specific 50^{th}, 75^{th} and 95^{th} percentiles.

Results

In this chapter there will be presented the main outcomes of the study.

At baseline and during the 2 weeks of detraining, participants used accelerometers in order to monitor their physical activity levels. Table 1 presents the analysed data, where it is possible to observe sedentary time, low-intensity physical activity (LIPA) and MVPA, all measured in total mean minutes/day, also the total mean steps per day. Compared to baseline, during detraining participants decreased their sedentary time by 20 min/day and MVPA by 5 min/day. Time spent in LIPA increased by 44 min, as well as the mean steps/day (+99). In the free living condition, 6 people met the criteria for being physically active according to ACSM

(at least 30 min of MVPA per day) (American College of Sports et al., 2018), while in detraining 5 people met this criteria.

Table 1: *Baseline and detraining characterization of sedentary and physical activity levels, all presented as total mean values.*

	Free living (baseline)	**Detraining**	**Mean change**
Sedentary time (min/day)	583 ± 59	563 ± 74	-20
LIPA (min/day)	243 ± 72	287 ± 79	44
MVPA (min/day)	30 ± 23	25 ± 22	-5
Steps/day	6974 ± 2603	7073 ± 2645	99

The sample consisted of 14 older men (n=8) and women (n=6) with a mean ± SD age of 77.2 ± 6.6 years old, height of 162 ± 0.1 cm and a baseline weight of 73.1 ± 8.2 kg. Most of participants (n=9) were overweight (25 kg/m2 – 30 kg/m2), 2 were obese (+30 kg/m2) and the remaining participants (n=3) were normal weighted (18.5 kg/m2 – 24,9 kg/m2). Table 2 shows the mean and standard deviation for all body composition variables (weight, fat and FFM, BMI, BMC, TBW, ECW, ICW and the ECW/ICW ratio), bioelectrical components (PhA, Xc, R), cardiorespiratory and strength outcomes ($VO_{2\ max}$, maximal strength in leg and bench press), for each assessment moment and changes in the same variables described before between moments of assessment.

After detraining, the PhA decreased by 4.34%, the Xc by 5.8% and the ICW compartment by 1.2%. Increases were found in ECW (3.23%) and in the ratio ECW/ICW (5.62%). No decreases were found in MS. The model used was adjusted for sex and all the results are represented in Table 2.When the model was adjusted for sex and age, the p-value found for PhA was p = 0.370.

***Table 2**: Changes between baseline and post-detraining regarding participant`s body composition, bioelectrical components and cardiorespiratory and strength fitness*

Variables	Baseline	Post-detraining	Mean change (%)	p-value*
Age (yrs)	77.2 ± 6.6			
Height (cm)	162 ± 0.1			
Weight (kg)	73.1 ± 8.2	73.4 ± 8.2	0.39	0.344
BMI (kg/m^2)	28 ± 3.9	28.1 ± 3.9	0.39	0.295
FM (kg)	23.6 ± 6.2	24 ± 6	1.02	0.303
FM (%)	32.7 ± 7.4	33 ± 7.2	0.95	0.243
FM Trunk (kg)	13.3 ± 3.2	13.6 ± 3.4	1.95	0.804
FFM (kg)	48.3 ± 7	48.5 ± 7.5	0.52	0.382
ALM (kg)	19.7 ± 3.7	19.7 ± 3.9	0.00	0.970
BMC (kg)	2.2 ± 0.5	2.2 ± 0.5	0.45	0.657
TBW (L)	39.5 ± 5.3	39.9 ± 5.7	0.91	0.542
ECW (L)	18.6 ± 3	19.2 ± 2.7	3.23*	**0.047***
ICW (L)	20.9 ± 2.9	20.7 ± 3.6	- 1.2*	**0.044***
ECW/ICW	0.89 ± 0.12	0.94 ± 0.13	5.62*	**0.011***
PhA (º)	5.8 ± 0.6	5.5 ± 0.6	- 4.34*	**0.017***
R (Ohms)	478 ± 53.6	472.4 ± 52.8	- 1.15	0.557
Xc (Ohms)	48.3 ± 8	45.5 ± 6.8	- 5.8*	**0.037***
$VO_{2\ max}$ (ml/kg/min)	25.1 ± 6	22.1 ± 4.6	- 11.9	0.391
Leg Press (kg)	131.9 ± 56.5	145.3 ± 51.7	10.1	0.992
Bench Press (kg)	62 ± 11.6	65.74 ± 9.05	6.8	0.166

Abbreviations: BMI, Body Mass Index; FM, Fat Mass; FFM, Fat Free Mass; ALM, Appendicular Lean Mass; BMC, Bone Mineral Content; TBW, Total Body Water; ECW, Extracellular Water; ICW, Intracellular Water; PhA, Phase Angle; R, Resistance; Xc, Reactance; $VO_{2\ max}$, maximum rate of oxygen consumption
Results are presented as mean ± s.d.

*Significantly different from baseline (p<0,05). Model adjusted to sex.

In Figure 5 are presented the PhA percentage changes for each participant. From the 14 participants, 10 decreased their PhA after 2 weeks of detraining. The changes observed in R (Ohm), Xc (Ohm) and PhA (°) are represented in Figure 6.

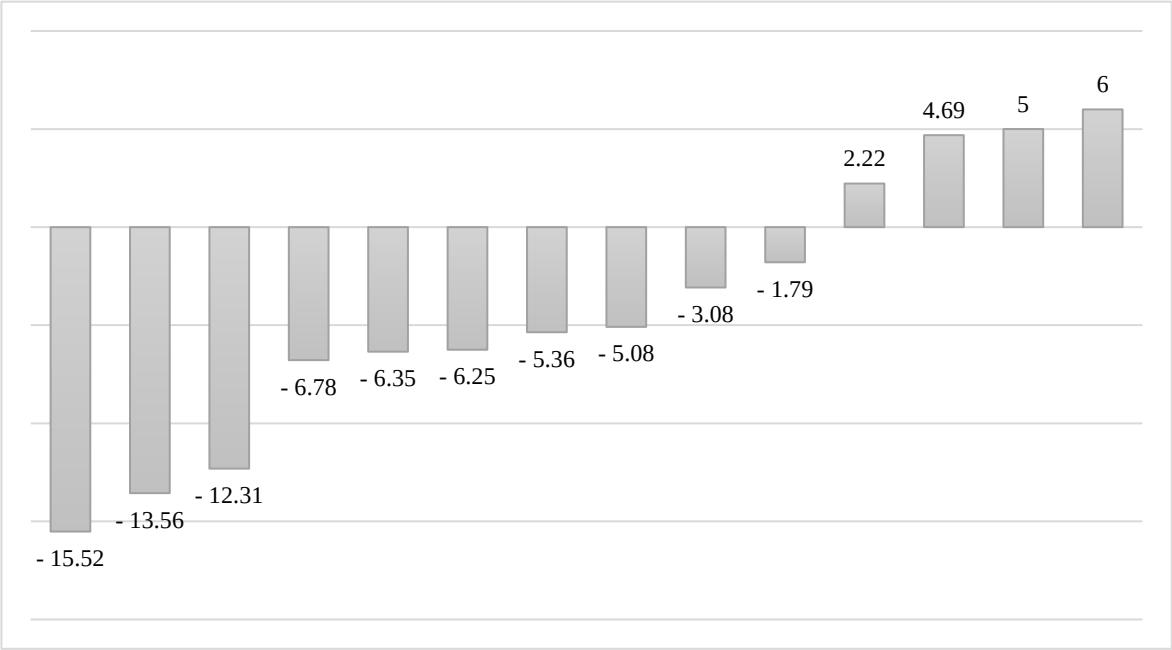

Figure 5: *Individual changes (%) of PhA after detraining*

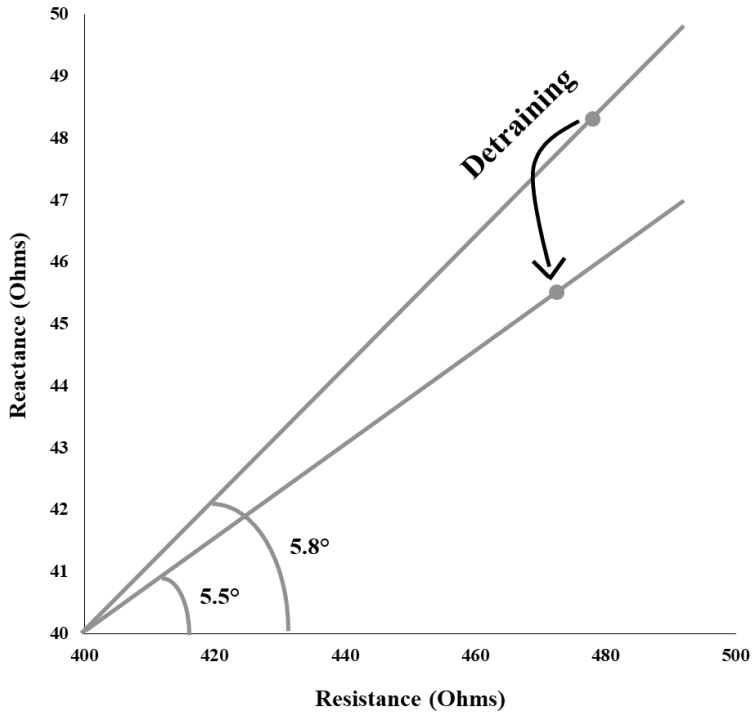

Figure 6: *Detraining changes in R (Ohm), Xc (Ohm) and PhA (°)*

Figure 7 represents the BIVA which used the mean R/H and the mean Xc/H adjusted to the reference population (Piccoli et al., 1995a). In both baseline and after detraining assessments, the mean values were placed in the third quadrant, within the 75th percentile. After detraining the vector moved down (more fluids, edema) and to the left (less soft tissue), with a tendency to move to the 90th percentile.

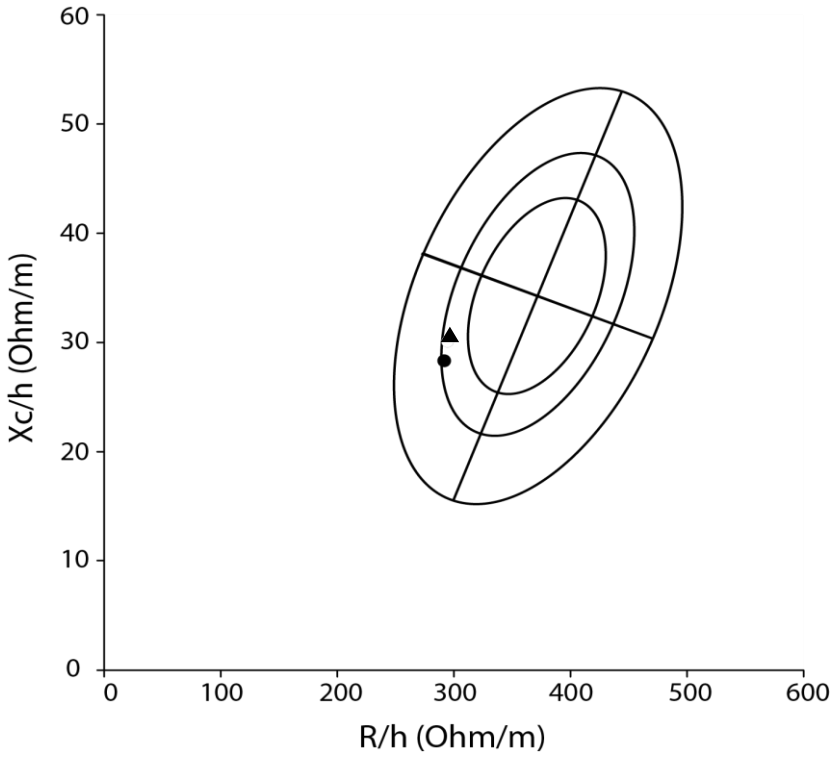

Figure 7: *BIVA changes between baseline (▲) and post-detraining (●)*

The Pearson correlation test was performed in order to find correlations between PhA and other variables (weight, BMI, %FM, FM of the trunk, ALM, all water compartments, $Vo_{2\ max}$, leg press and bench press strength). At baseline, PhA was negatively correlate with ECW ($r = - 0.55$, $p<0.05$) and the ECW/ICW ratio ($r = - 0.99$, $p<0.05$). At post-detraining, PhA had a positive correlation with ICW ($r = 0.63$, $p<0.05$) and bench press strength ($r = 0.76$, $p<0.05$), and a negative correlation with the ECW/ICW ratio ($r = - 0.99$, $p<0.05$). When considering only mean differences between baseline and post detraining, PhA was negatively correlated

with ECW (r = - 0.81, p<0.05) and ECW/ICW ratio (r = - 0.97, p<0.05), and positively correlated with ICW (r = 0.89, p<0.05) (Table 3).

Table 3: *Correlation coefficients between PhA and other variables*

	PhA Baseline [1]	**PhA Post-Detraining [2]**	**Dif PhA [3]**
Weight	- 0.23	0.06	- 0.39
BMI	- 0.08	- 0.16	- 0.40
%FM	- 0.26	- 0.41	- 0.17
FM Trunk	- 0.25	- 0.21	- 0.46
FFM	0.02	0.47	0.05
ALM	0.07	- 0.20	- 0.22
TBW	- 0.14	0.31	0.41
ECW	**- 0.55***	- 0.21	**- 0.81****
ICW	0.32	**0.63***	**0.89****
ECW/ICW	**- 0.99****	**- 0.99****	**- 0.97****
$VO_{2\ max}$	0.11	0.00	- 0.19
Leg Press	0.46	0.46	0.39
Bench Press	0.08	**0.76****	-0.04

* Significant correlation, considering p<0,05 (two-tailed)

** Significant correlation, considering p<0,01 (two-tailed)

[1] Correlated with the variables in the left column assessed at baseline

[2] Correlated with the variables in the left column assessed at post-detraining

[3] Correlated with the variables in the left column calculated as the difference between the two moments

Discussion

The main purpose of this thesis was to analyse the physiologic effects of a 2-week detraining in older trained adults and also to observe if there is a relation between PhA and MS, in a randomized crossover trial where the participants assume themselves as controls. We found

that 2 weeks of detraining cause a significant decrease in PhA, regardless of sex. However, the same is not applicable for MS. Also, considering the relation between PhA and MS, only PhA in post-detraining had a significant positive correlation with MS of upper limbs (bench press).

1. Phase Angle (PhA)

According to the results, our sample which was comprised by 14 trained older people (6 females), presented a mean PhA at baseline of 5.8º ± 0.6. When considering men and women separately, men had a mean PhA of 5.8º ± 0.7 and women of 5.7º ± 0.6. Comparing to existing evidence, these mean values of PhA showed to be higher than the values observed in similar studies which also worked with healthy elderly, however not in the same condition of training. Buffa et al. (2003) used a sample of healthy individuals and both women and men, aged between 60 and 89 years old, revealed mean values of PhA (men = Δ 5.63º; women = Δ 5.46º) lower than the values observed in the present study (Buffa et al., 2003). The same is valid for the studies of Kyle et al. (2001) (men = Δ 5.33º; women = Δ 4.93º) and Basile et al. (2014) (Δ 5.1º) in which they both had samples of healthy older people aged 65 years old or more (Basile et al., 2014; Kyle, Genton, Slosman, & Pichard, 2001). This may be due to the fact that the population addressed in our study consists of active and trained individuals. Still, this study revealed lower values, in both women and men, when compared to a study which assessed body composition in healthy elderly aged 65 or more years old (men = Δ 6.2º; women = Δ 5.9º) (Saragat et al., 2014). The PhA values of our participants in post-detraining decreased for both older men (5.7º ± 0.7) and women (5.3º ± 0.4), and became closer to the values observed in most of the studies mentioned above in which the training condition did not exist.

Considering studies which used healthy adult populations, the PhA values observed in our study in both baseline and after detraining were lower than the values found by Kyle et al. (2012) (men = Δ 7.55º; women = Δ 6.5º) authors and Selberg & Selberg (2002) (Δ 6.6º) (Kyle,

Soundar, Genton, & Pichard, 2012; Selberg & Selberg, 2002). This seems logic since PhA is negatively related with aging, starting in adulthood (Bosy-Westphal et al., 2006). Lower values suggest a more negative cellular health, less integrity of cell membranes and worse cellular function (Selberg & Selberg, 2002). All of this provides information on oxidative stress, inflammatory biomarkers and, therefore, sarcopenia, which is typical to exist in older individuals (Basile et al., 2014; Norman et al., 2012; Tomeleri et al., 2018). In our analysis, after adjusting the model for the variable age, the PhA had a non-significant p-value of 0.370, which may be related to the wide range of our participants` ages (range = 20 years old). As authors have observed, age is of great importance in PhA variations, so this was the expected outcome (Barbosa-Silva et al., 2005; Bosy-Westphal et al., 2006).

Bosy-Westphal et al. found that PhA was significantly greater in men than in women when considering a BMI between 18.5–25 or >35 kg/m2 and excluding subjects older than 70 (Bosy-Westphal et al., 2006). Most of the studies mentioned above also showed PhA values greater in men compared to women, in older populations (Buffa et al., 2003; Kyle et al., 2001; Saragat et al., 2014). In the present study, similar to these findings, this was also observed. At baseline, men had a mean PhA higher than women (men: $5.8^{o} \pm 0.7$ < women: $5.7^{o} \pm 0.6$), and remained higher after detraining (men $5.7^{o} \pm 0.7$ > women $5.3^{o} \pm 0.4$). This means that after two weeks of detraining, the magnitude of the decrease of PhA was higher in women (Δ = - 7.5%) compared to men (Δ = - 1.9%). Despite the differences between men and women, our results showed that PhA decreased in either sexes after a period of training cessation.

As mentioned before the consequences of detraining rely on specific characteristics, such as its duration, frequency and intensity (Fleck, 1994; Mujika & Padilla, 2000). After a period of detraining, it is possible to observe losses in the endurance capabilities, bioelectrical properties and in the skeletal muscle power (Fleck, 1994). According to this, we found reductions in PhA values (Δ = - 4.34%) and Xc (Δ = - 5.8%). We also observed an interesting

tendency to decreases in VO_{2max} (Δ = - 11.9%), which would probably have a major relevance if the sample size was greater.

There is little evidence about the effects of detraining on PhA and other bioelectrical properties. So far, one study found that an interruption in the training routine may cause a significant decline in the PhA, which is greater than the gains obtained with previous training, and which can only be recovered partially with retraining (Dos Santos et al., 2016). Similar to this findings, in trained older people, we found that PhA had a mean percentage change of -4.32% after 2 weeks of detraining. Santos et al. (2016) also observed a significant increase in R, however this is not consistent with our findings.

As mentioned before, PhA is a biomarker directly affected by changes in the amount and quality of soft tissue mass and hydration, since it necessarily depends on Xc and R (Bosy-Westphal et al., 2006; Tomeleri et al., 2017). PhA tends to decrease as a consequence of decreases in Xc, which may be caused by the decline of body water proportions and changes in body composition (increased subcutaneous, intraabdominal and interstitial fat and decreases in FFM) (Ackmann & Seitz, 1984; Baumgartner et al., 1988; Norman et al., 2012). This consents with our results, since there was a mean drop of both Xc and PhA. When analysing other body composition components, there was a tendency to increases in weight (Δ = 0.39%), BMI (Δ = 0.39%), FM in kg (Δ = 1.02%), FM % (Δ = 0.95%) and FM of the trunk (Δ = 1.95%). These results concur with the findings mentioned above and may, in part, explain changes in PhA.

When considering body water compartments, both ECW and the ECW/ICW ratio increased after detraining (**Δ =** 3.23% for ECW and **Δ =** 5.62 for the ECW/ICW ratio). Besides that, our BIVA outcomes (detailed forward in this topic) suggests there was an increase of hydration and a decrease of body cell integrity and mass. This concurs with other authors findings, proposing that ECW and the ECW/ICW ratio are usually increased in the presence of

edema, malnutrition and/or disease, which seems to be due to a shift from intra to extracellular space (Kyle et al., 2004; Malbrain et al., 2014). Therefore, decreases in ICW also reflect changes in the nutritional status, in particular loss of BCM (C. P. Earthman et al., 2000; Yoon et al., 2018), which sustains the decreases found in the ICW compartments of our participants (**Δ** = - 1.2%).

We suggest that the ECW/ICW ratio should be determinant for the PhA variation, similarly to other studies (Gonzalez et al., 2016). Authors have found that individuals with lower values of PhA have a tendency to present less ICW, an expansion of ECW and a greater ECW/ICW ratio, due to low Xc - less amount of cell membranes and smaller cell size (Buffa et al., 2013; Chertow et al., 1995; Foster & Lukaski, 1996; Guo et al., 1996; Malbrain et al., 2014). In the present investigation, after detraining, PhA decrease by 4.34% and the ECW/ICW ratio increased by 5.62%. Also, from our correlation analysis we observed that when PhA decreases there is a tendency for ECW and the ECW/ICW ratio to increase, while ICW tends to decrease.

Another possible explanation for the decrease of PhA would be BCM. This component is essentially the FFM with no bone mineral mass and extracellular water (Oliveira et al., 2010) and is considered one of the most metabolically active compartments (Moore & Boyden, 1963; Oliveira et al., 2010). Is directly related with Xc and PhA (Oliveira et al., 2010) and when the integrity of body cells is compromised, the capacitance drops, which directly affects PhA (Selberg & Selberg, 2002). Thus, BCM, Xc and PhA are related and seem to be useful biomarkers for the nutritional status, specifically in patients. BCM was not directly measured in this study but an interpretation of it was possible through the BIVA.

As mentioned before, BIVA uses R and Xc to access fluid status - total body water -, and BCM (Bosy-Westphal et al., 2005; Piccoli et al., 1996; Walter-Kroker et al., 2011). Our results showed that in both baseline and post-detraining assessments, the mean values were

placed in the third quadrant. Before detraining, these values were placed within the 75th percentile, and after detraining the vector moved down and to the left, still remaining in the 75th percentile but showing a clear tendency towards the 95th percentile. The 75th percentile is usually where the values of healthy population are located (Norman et al., 2012; Piccoli et al., 1998), as within the 95th percentile are frequently values considered abnormal (Bosy-Westphal et al., 2005; Dorhofer & Pirlich, 2005; Walter-Kroker et al., 2011). This means that after detraining there was a mean decrease of R due to an increase of hydration, which causes edema and fluid overloading. Also, as expected from lack of training, there was a decrease of Xc which may be explained by a decline of cell integrity and BCM. This concurs with the findings of Dos Santos et al. (2016) which also observed a decrease in Xc that could be related to cell death or increased fragility and proposed that this could be a possible explanation for the decrease of PhA (Dos Santos et al., 2016).

2. Muscle Strength (MS)

Results showed that there were no significant changes in MS after 2 weeks of detraining. Our results appear to be unexpected, in fact, the mean percentage change was positive for both leg press ($\Delta = 10.1\%$) and bench press ($\Delta = 6.8\%$).

Several studies found contradictory results when observing the effects of detraining in muscle quality in trained individuals, and evidence shows that the losses associated to detraining depend on the duration of this interruption (Tokmakidis et al., 2009). Opposable to our findings, interventions also performed with older people observed that after a period of detraining (12 weeks) both maximal MS and hypertrophy levels significantly decreased, however these values did not regress to the baseline values previous to the participation in a training program (Tokmakidis et al., 2009). Other studies also measured MS (isometric and dynamic) in older people and found that after 8, 24 and 48 weeks of detraining, there was a

significant decrease in MS (Elliott, Sale, & Cable, 2002; Fatouros et al., 2005; Henwood & Taaffe, 2008).

A different argument was used by other authors. After long periods of detraining (from 12 to 31 weeks) some authors measured MS and muscle volume (MV) (cross-sectional areas) and found that there was a retention of MS but a significant decrease of MV (Correa et al., 2013; Hakkinen, Alen, Kallinen, Newton, & Kraemer, 2000; Ivey et al., 2000; Taaffe & Marcus, 1997; Van Roie et al., 2017). It is common to find weak correlations between changes in MV and MS, since there is usually a loss of muscle mass but retention of MS. Also, when considering training, the same is observed: similar muscle growth for different training loads, but divergent results in strength (Buckner et al., 2016). Because of these findings, authors believe that other factors apart from muscle mass contribute to strength levels, therefore the preservation of strength after detraining seems to be caused by a retention of neural adaptations and these may play a greater role in MS than muscle hypertrophy. This evidence, similar to ours, suggests that older people have the same muscle adaptability as younger adults and proposes that short periods of the cessation of training will not have a deleterious effect on MS (Taaffe & Marcus, 1997).

Following the same line, Sforzo et al. (1995) found that older people are quite resilient to detraining when considering cardiovascular fitness, and retain MS for at least 5 weeks after an interruption of formal exercise (Sforzo, McManis, Black, Luniewski, & Scriber, 1995). This may be an important finding since it is very common for older adults to voluntary engage in training cessation, whether is due to the participation in extended holidays, volunteering or family commitments (Henwood & Taaffe, 2008). An occasional missed exercise session or temporary cessation of habitual exercise would not be a major problem. However, it is still important to promote the participation in regular exercise among older populations and make

them aware of the benefits of being active and of the ease with which they may restart an exercise program after ceasing it (Sforzo et al., 1995).

Apart from these findings there may be other explanations for the fact that our study did not find significant results in MS after 2 weeks detraining. All the studies mentioned before used periods of detraining longer than 2 weeks and in most of them MS still had no significant changes. Similar to these, in the present study this short period of detraining was not enough to find significant decreases in MS. Also, although the participants refrained from exercise during the 2 week-detraining, there was most likely a resilience to decrease the levels of physical activity during their leisure time and the cessation of structured exercise alone may not have been sufficient to reduce MS.

In addition, some of these studies measured muscle cross-sectional areas, which was not performed in our study. However, through DXA it was possible to measure ALM and FFM, which are great predictors of lean mass, and we found that any of these variables suffer significant changes after detraining. Considering these findings it is likely that MV did not change after detraining.

A different possible explanation for the discrepancy between our results and findings by other authors is that strength adaptations may be influenced by factors such as gender, age, body composition, conditioning level, also by the characteristics and magnitude of previous training programs (e.g. frequency, intensity and type of exercise, training volume) and detraining period (particularly, its duration and the level and intensity of physical activity performed during detraining) (Correa et al., 2013; Esain et al., 2018; Kraemer & Ratamess, 2004). Among these factors, the results of this study may have been affected, specially, by the type of test used to assess MS. This is crucial since strength tests differ in movement patterns, contractile speeds, and neuromuscular qualities assessed (Elliott et al., 2002). Also, this type of testing may be of questionable accuracy and reliability since it is very sensible to the

individual`s mood, social or personal factors, experience and learning of the testing movements, and even tiredness or mental fatigue (Amundsen, 1990; Hopkins, 2000). Motivation and willingness, specifically, constitute an important role, as participants may had an attempt to improve their previous performance, although we did not inform them about the results (Brotons-Gil, Garcia-Vaquero, Peco-Gonzalez, & Vera-Garcia, 2013; Hopkins, 2000).

Furthermore, several studies using submaximal and maximal testing have shown that a learning effect may occur because of test familiarization, especially when the individuals are "naive" in relation to the testing concerned (never experimented it before) (Brotons-Gil et al., 2013; Gibbons, Fruchter, Sloan, & Levy, 2001; Wu, Sanderson, & Bittner, 2003). A study concluded that the initial learning effect is maintained during a 2-month period (Wu et al., 2003), however it is still not well known for how long this initial effect persists and how it differs according to the type of testing. Because there were only 2 weeks between the first and the last evaluation, there was most likely a learning effect of the movements which may, considerably, explain the unexpected increase of strength levels.

Considering all the issues exposed above, it is not possible for us to find a plausible reason to explain these findings given all the factors impacting this form of testing.

3. Phase angle (PhA) vs muscle strength (MS)

It has been observed by some authors that, independently of factors as age, PhA is positively correlated with muscle function, which includes MS, muscle mass and capability to perform daily activities (Basile et al., 2014; Beberashvili et al., 2014; Norman et al., 2012; Norman et al., 2010). Higher values of PhA seem to exist in parallel with higher values of MS, which suggests that PhA may be a useful predictor of muscle dysfunction (Basile et al., 2014; Beberashvili et al., 2014). Similar to this findings, we observed that individuals who had higher PhA values after detraining, showed higher bench press strength in the same measurement

moment ($p<0.05$). Our results may differ from former findings because of the method used to measure MS – most of the studies which intended to observe the relation between PhA and MS used the 1RM method or hand-grip strength, while in the present study participants were evaluated under isometric conditions (Basile et al., 2014; Beberashvili et al., 2014; Norman et al., 2012; Norman et al., 2010).

Authors which studied the effects of training in PhA and MS and/or muscle mass, or MS alone, found that they both increase after the participation in a training program (Cannon, Kay, Tarpenning, & Marino, 2007; Fatouros et al., 2005; Faulkner & Brooks, 1995; Fukuda et al., 2016; Souza et al., 2017). Due to this findings it would be expected that after a period of detraining the opposite trend would occur – decreases in PhA, muscle mass and strength. However, only one study has already observed the effects of detraining in both PhA and MS (Dos Santos et al., 2016). Dos Santos et al. found that an interruption of 12 weeks in the training routine of older women led to a significant decline in PhA, an increase in R and a decrease in MS (1RM in chest press and leg extension) (Dos Santos et al., 2016). Similar to this study, after 2 weeks of detraining we found a significant decrease of PhA but not in R or MS. When considering the mean changes with detraining, we also found no significant correlation between PhA and MS.

During this intervention, we always focused on protecting the integrity of our participants, so we did not expose them to extreme decreases in physical activity. Time spent in MVPA decreased during detraining, which leads us to confirm that the participants, in deed, did not engage in any structured exercise sessions. Also, the participants were advised to reduce their physical activity levels in leisure time. However, we observed that LIPA increased and sedentary time decreased during detraining, which was possibly a mechanism to compensate the inexistence of the structured exercise sessions during detraining. This may have limited the

magnitude and significance of our results. A greater restriction in physical activity would be necessary in order to get more reliable outcomes.

Furthermore, at baseline, not all participants met the criteria for being physically active, according to ACSM. Nonetheless, it is important to mention that some of the participants perform structured sessions in a swimming pool, with the duration of at least 45 minutes. The accelerometers do not record data during this time, plus the time spent bathing and dressing up. Also, the participants perform structured exercise sessions which include strength exercises performed on machines and in a sitting position. The time spent doing this type of exercises may have been accounted as sedentary time, which does not correspond to the reality (Sardinha, Magalhaes, Santos, & Judice, 2017). Therefore, during the free living condition, participants` physical activity may have been underestimated, while sedentary time may have been overestimated.

Limitations

This study showed some limitations which need to be addressed, despite the positive findings.

Other similar studies used larger samples which allowed them to generalize their results. In the present study this was not possible because of the small sample size (n=14).

There was, most likely, a lack of adherence from our participants to decrease their physical activity levels in leisure time. A greater restriction in physical activity would be necessary in order to get more reliable outcomes.

The method used to measure physical activity and sedentary time (accelerometers), did not measure swimming pool exercise sessions and may have accounted exercise in a sitting position as sedentary time, which does not correspond to the reality. This most likely caused

an underestimation of physical activity and overestimation of sedentary time, during the free living condition.

Another limitation of this study concerns the assessment of MS. The method used, which was the isometric leg press and bench press, is highly sensitive to learning and experience, as it is to personal feelings, tiredness and motivation. The participants only became acquainted with the equipment and the movements involved in the testing on the first assessment performed at baseline. They should have had a period of familiarization with the equipments and the testing previous to the beginning of the first evaluation session. This would probably avoid the learning effect or, at least, attenuate the magnitude of its effects.

Conclusion

In healthy physically active older adults, 2 weeks of detraining significantly decreased PhA, suggesting that this is a more sensitive marker, when compared to a number of other outcomes, that did not differed between moments. No changes were found for MS from detraining and only PhA in post-detraining had a significant positive correlation with MS of upper limbs (bench press).

Since we found a major effect on PhA, we suggest that this may be considered as a marker sensitive to training/detraining in older adults that may provide information about the individual`s participation in exercise programs and its efficacy.

This findings highlight the need for older populations to regularly engage in physical activity and exercise sessions and avoid long periods of inactivity, in order to prevent the aggravation of health situations due to body composition changes.

Future work

More studies concerning the role of detraining on PhA and MS are needed, as to the authors knowledge, there is only one besides ours. Although this study found only a slight relation between PhA and MS, more studies should further explore this potential association. Also, additionally to MS, muscle mass should be assessed in order to completely understand if muscle function, in its whole, suffers transformations after a period of detraining. A greater attention should be given when accessing MS, in order to reduce bias. It is important to have a period of familiarization with the equipments and the testing previous to the beginning of the intervention, so the learning effect can be attenuate or even non-existing.

Since this study observed the effects of short-term detraining, it would be interesting to perform a longer detraining, in order to understand the long-term effects on PhA.

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APPENDIX A – Exclusion Criteria Questionnaire

QUESTIONÁRIO DE AVALIAÇÃO

Nome: _____

Género:. Feminino □ Masculino □ Data: ___/___/ ___ Data de nascimento: ___/___/___

Telefone: _____ Peso (kg): _____ Altura (m): _____

Fuma? Sim □ Quantos cigarros por dia? _____

Não □

DOENÇAS CRÓNICAS

Apresenta alguma das seguintes doenças?

Doenças cardiovasculares:

- □ Hipertensão arterial
- □ Enfarte do miocárdio
- □ Angina do peito
- □ AVC

Outra(s): _____

- □ Colesterol elevado
- □ Osteoporose

Outra(s): _____

Doenças Renais □ Doenças Pulmonares (Ex: Asma) □

Qual/Quais: _____

Doenças metabólicas:

- □ Artrite/Artrose
- □ Cancro
- □ Anemia
- □ Diabetes

Outra(s): _____

Teve alguma lesão nos últimos 6 meses? Sim □ Não □

Se sim, que lesão? _____

Ocorrência de Fraturas

N.º total de fraturas que sofreu ao longo da vida: _____ Local ósseo das fraturas:

_____

Ocorrência de quedas (últimos 12 meses)

Quantas vezes caiu no último ano? _____

Ocorrência das quedas:

☐ A realizar uma tarefa usual

☐ A realizar uma tarefa excepcionais ou de grande dificuldade

☐ A realizar uma actividade física supervisionada por um professor

Medicação

Alguma vez tomou algum medicamento mais de 6 meses? Que outros medicamentos toma atualmente? (* indicar para que doença o medicamento foi prescrito)

*	Medicamento	Ano de início	N.º de anos

Observações:

Saúde e Incapacidade Física

Apresenta algum dos seguintes problemas de saúde?

	Sim	Não
1. Incontinência urinária (perda de urina)	☐	☐
2. Tonturas frequentes	☐	☐
3. Problemas nos pés (inflamações, calos, etc)	☐	☐
4. Problemas de visão (não reconhece uma pessoa a quatro metros de distância mesmo usando óculos ou lentes de contacto).	☐	☐
5. Problemas da audição (não consegue seguir uma conversa de um grupo de quatro pessoas mesmo com aparelho auditivo)	☐	☐
6. Problemas de equilíbrio (algumas vezes tem dificuldade em equilibrar-se)	☐	☐

Quantas vezes foi ao médico no último mês? _____

Quantas vezes foi ou permaneceu no hospital nos últimos 6 meses? _____

Considera que a sua saúde é: Muito má ☐ Má ☐ Razoável ☐ Boa ☐ Excelente ☐

Notou perda de peso involuntária nos últimos 12 meses? ☐ Não ☐ Sim (peso inicial e peso perdido)

Tem tido falta de apetite? ☐ Não ☐ Sim

No último mês sentiu que tinha muito pouca energia para as coisas que pretendia realizar? ☐ Não ☐ Sim

Ocupações do tempo livre

Quais das seguintes atividades gosta de praticar no seu tempo livre?

- □ Ver televisão?
- □ Jogar computador
- □ Ler um livro/jornais/revistas
- □ Ouvir música
- □ Jogos de tabuleiro
- □ Jogar às cartas
- □ Fazer sudoku

Outras: _____

Calendarização da Intervenção

Relativamente à Calendarização da Intervenção (pode assinalar mais do que 1 opção):

- □ Não posso realizar a intervenção se esta for durante o mês de Janeiro
- □ Não posso realizar a intervenção se esta for durante o mês de Fevereiro
- □ Não posso realizar a intervenção se esta for durante o mês de Março
- □ Não posso realizar a intervenção se esta for durante o mês de Abril
- □ Não posso realizar a intervenção se esta for durante o mês de Maio
- □ Não tenho preferência ou restrições relativamente a nenhuma das datas

Já participou em outro(s) estudo(s)?

- □ Sim. Se sim, qual? _____
- □ Não

Obrigado pela sua colaboração!

APPENDIX B - Informed Consent

CONSENTIMENTO INFORMADO LIVRE E ESCLARECIDO

Título do projeto: Efeitos da Interrupção do Comportamento Sedentário e da Inatividade Física na Resposta Pós-prandial da Glicémia, na Sensibilidade à Insulina, na Flexibilidade Metabólica e no ângulo de fase

Pessoa responsável pelo projeto: Professor Doutor Luís Bettencourt Sardinha

Instituição de acolhimento: Faculdade de Motricidade Humana – Universidade de Lisboa

Este documento, designado **Consentimento, Informado, Livre e Esclarecido**, contém informação importante em relação ao estudo para o qual foi abordado/a, bem como o que esperar se decidir participar no mesmo. Leia atentamente toda a informação aqui contida. Deve sentir-se inteiramente livre para colocar qualquer questão, assim como para discutir com terceiros (amigos, familiares) a decisão da sua participação neste estudo.

Este estudo visa avaliar os efeitos da interrupção do comportamento sedentário e do destreino na resposta pós-prandial da glicémia, na sensibilidade à insulina e na flexibilidade metabólica em idosos treinados.

Para tal, ser-lhe-á solicitada a sua participação **durante um mês**, no qual será sujeito, com acompanhamento de pessoas especializadas (enfermeiro e médico). No final deste documento, é disponibilizada uma tabela que ilustra os vários procedimentos que serão necessários realizar e que se encontram descritos, em texto, de seguida:

A **avaliação inicial** inclui seis procedimentos, a realizar em dois dias distintos: DIA 1 - **DXA** (densitometria raio-x de dupla energia) para avaliação da composição corporal e **bioimpedância**; **avaliação da flexibilidade metabólica** (medição do metabolismo de repouso durante meia hora com recolha de sangue, ingestão de uma refeição padrão; medição do metabolismo de repouso durante 2 horas, com recolha de sangue aos 60 e 120 minutos); DIA 2 - **prova de esforço** em passadeira para avaliação da aptidão cardiorespiratória; **avaliação da força muscular** (testes com sensores de força); preenchimento de **questionários** relativos aos hábitos alimentares, qualidade de sono, hábitos de atividade física e estado de saúde geral;

No **primeiro dia de intervenção, após os procedimentos da avaliação inicial** (DIA 3) será realizado um teste de tolerância oral à glucose (OGTT-2h) com recolha sanguínea em jejum e aos 120 minutos. Seguidamente, serão administradas duas refeições padrão (pequeno – almoço e almoço) e aplicado aleatoriamente um dos protocolos:

- **Protocolo A** consiste na manutenção de um comportamento sedentário durante 7 horas (3 horas durante a manhã + 4 horas durante a tarde);
- **Protocolo B** serão feitas interrupções de 2 minutos a cada meia hora no comportamento sedentário (3 horas durante a manhã + 4 horas durante a tarde). As interrupções consistirão na realização de um exercício, de dois possíveis: sentar e levantar da cadeira ou descer e subir

escadas. Os exercícios serão realizados alternadamente entre os diferentes períodos de interrupção do comportamento sedentário e a ordem de realização dos mesmos será definida pelas alunas que acompanham o protocolo.

No dia seguinte à aplicação de um protocolo (DIA 4) será realizado um OGTT (de manhã).

Após 4 dias da realização do primeiro protocolo, será aplicado o protocolo A ou B (exemplo: se no primeiro momento realizou o A, 4 dias após irá realizar o B).

Uma vez realizados ambos os protocolos, será sujeito a um **período de inatividade física de 15 dias**. Durante este tempo, solicita-se que não frequente as sessões de exercício que normalmente realiza na Faculdade de Motricidade Humana e fora desta, e que minimize os níveis de atividade física. Simultaneamente, ser-lhe-á solicitado o uso do acelerómetro em dois dias úteis e um dia de fim-de-semana, em cada uma dessas duas semanas.

Posteriormente, serão repetidos os procedimentos A e B, conforme o acima descrito, para comparar a resposta a vários parâmetros, após duas semanas de destreino.

A **avaliação final** consistirá na realização dos procedimentos feitos na avaliação inicial e será realizada após a repetição de ambos os protocolos.

A sua participação é voluntária e pode recusar-se a participar. Caso decida participar neste estudo é importante ter conhecimento que pode desistir a qualquer momento, sem qualquer tipo de consequência para si. No caso de decidir abandonar o estudo, a sua relação com a Faculdade de Motricidade Humana (FMH) não será afetada. Se for o caso, o seu estatuto enquanto estudante ou funcionário da FMH será mantido e não sofrerá nenhuma consequência da sua não-participação ou desistência.

Através deste estudo terá disponível, para seu conhecimento, informação detalhada relativa à sua condição cardiorrespiratória, composição corporal, valores glicémicos, sensibilidade à insulina, valores lipídicos e saúde metabólica. Adicionalmente, será informado/a de estratégias para interrupção do comportamento sedentário. Porém, este estudo não está isento de riscos, nomeadamente durante a prova de esforço, ainda que os mesmos sejam reduzidos devido à presença de um médico. Para além disso, poderá sentir algum desconforto decorrente das diversas colheitas de sangue, pelo que será aplicado um creme analgésico durante o período da recolha e ser-lhe-á fornecido um creme para colocar nos dias seguintes de forma a evitar o aparecimento de hematomas, como por exemplo nódoas negras.

Todos os dados deste estudo serão recolhidos, tratados e guardados (na FMH-UL e na Associação Protetora dos Diabéticos de Portugal) em regime de confidencialidade. Serão guardadas amostras de sangue suas, devidamente codificadas (garantido a confidencialidade dos dados), na FMH-UL destinando-se meramente para efeitos de investigação. Os resultados do estudo serão divulgados nas dissertações finais das alunas de mestrado e os mesmos ser-lhe-ão disponibilizados.

Em caso de dúvida ou situação de urgência, deverá ser contactada uma das alunas: Inês Correia (926149130), Júlia Lopes (916951976), ou Sofia Freitas (910357965).

Li (ou alguém leu para mim) o presente documento e estou consciente do que esperar quanto à minha participação no estudo: **Efeitos da Interrupção do Comportamento Sedentário e do Destreino na Resposta Pós-prandial da Glicémia, na Sensibilidade à Insulina, na Flexibilidade Metabólica e no**

Ângulo de Fase em Idosos Treinados. Tive a oportunidade de colocar todas as questões e as respostas esclareceram todas as minhas dúvidas. Assim, aceito voluntariamente participar neste estudo. Foi-me dada uma cópia deste documento.

Desde já, agradecemos o facto de se disponibilizar a ler este consentimento e, se assim o decidir, a participar no nosso projeto. Obrigado pela sua atenção!

Calendarização individual (calendarização tipo a adaptar para cada participante)

Dia	Horas	Local	O que fazer	Observações
8 Maio	A definir	Pavilhão Lord - FMH	Avaliação inicial	Equipado com fato de treino e ténis
12 e 13 Maio	**Não realizar AF vigorosa; no entanto, pode fazer caminhada leve; EVITAR CONSUMO DE BEBIDAS ALCÓOLICAS E BEBIDAS COM CAFEÍNA**			
14 Maio	7:30 – 10:30	Pavilhão Lord - FMH	Avaliação inicial	Em jejum e sem metais (ex: brincos)
14 e 15 Maio	**Não realizar AF vigorosa; no entanto, pode fazer caminhada leve; EVITAR CONSUMO DE BEBIDAS ALCÓOLICAS E BEBIDAS COM CAFEÍNA**			
16 Maio	8:00 – 18:00	Pavilhão Lord - FMH	OGTT + Protocolo 1	Vir em jejum
17 Maio	8:00 – 10:00	Pavilhão Lord - FMH	OGTT	Vir em jejum
19 e 20 Maio	**Não realizar AF vigorosa; no entanto, pode fazer caminhada leve; EVITAR CONSUMO DE BEBIDAS ALCÓOLICAS E BEBIDAS COM CAFEÍNA**			
21 Maio	8:00 – 18:00	Pavilhão Lord - FMH	OGTT + Protocolo 2	Vir em jejum
22 Maio	8:00 – 10:00	Pavilhão Lord - FMH	OGTT	Vir em jejum
23 Maio a 5 Junho	**DESTREINO:** Não realizar aulas estruturadas de exercício (ginásio ou piscina); **minimizar** atividade física (caminhadas, por exemplo); usar acelerómetro todos os dias; nos **dias 4 e 5 de Junho → EVITAR CONSUMO DE BEBIDAS ALCÓOLICAS E BEBIDAS COM CAFEÍNA**			
6 Junho	8:00 – 18:00	Pavilhão Lord - FMH	OGTT + Protocolo 1	Vir em jejum
7 Junho	8:00 – 10:00	Pavilhão Lord - FMH	OGTT	Vir em jejum
9 e 10 Junho	**Não realizar AF vigorosa; no entanto, pode fazer caminhada leve; EVITAR CONSUMO DE BEBIDAS ALCÓOLICAS E BEBIDAS COM CAFEÍNA**			
11 Junho	8:00 – 18:00	Pavilhão Lord - FMH	OGTT + Protocolo 2	Vir em jejum
12 Junho	8:00 – 10:00	Pavilhão Lord - FMH	OGTT	Vir em jejum
13 Junho	**Não realizar AF vigorosa; no entanto, pode fazer caminhada leve; EVITAR CONSUMO DE BEBIDAS ALCÓOLICAS E BEBIDAS COM CAFEÍNA**			
14 Junho	7:30 – 10:30	Pavilhão Lord - FMH	Avaliação Final	Em jejum e sem metais (ex: brincos)
20 Junho	A definir	Pavilhão Lord - FMH	Avaliação Final	Equipado com fato de treino e ténis

OGTT – teste de tolerância oral à glucose (tempo de realização 2h com duas colheitas de sangue)

Protocolo 1 e 2 – um dia sentado + um dia sentado com interrupções (10:30h – 18:00h)

Avaliação inicial e final

- **Prova de esforço** (avaliação da capacidade cardiorrespiratória)
- **Avaliação da força muscular**
- **Densitometria óssea** (avaliação da composição corporal)
- **Bioimpedância** (avaliação da composição corporal)
- **Saúde metabólica (com recolha de sangue)**
- **Questionários**

Assinatura do Consentimento Informado, Livre e Esclarecido

Li (ou alguém leu para mim) o presente documento e estou consciente do que esperar quanto à minha participação no estudo **Efeitos da Interrupção do Comportamento Sedentário e do Destreino na Resposta Pós-prandial da Glicémia, na Sensibilidade à Insulina, na Flexibilidade Metabólica e no Ângulo de Fase em Idosos Treinados**. Tive a oportunidade de colocar todas as questões e as respostas esclareceram todas as minhas dúvidas. Assim, aceito voluntariamente participar neste estudo. Foi-me dada uma cópia deste documento.

Nome do participante	**Assinatura do participante**

Data

Investigador/Equipa de Investigação

Os aspetos mais importantes deste estudo foram explicados ao participante ou ao seu representante, antes de solicitar a sua assinatura. Uma cópia deste documento ser-lhe-á fornecida.

Nome da pessoa que obtém o consentimento	**Assinatura da pessoa que obtém o consentimento**

Data